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Facilitative Effects on Performance Following Modification of Circadian Rhythms

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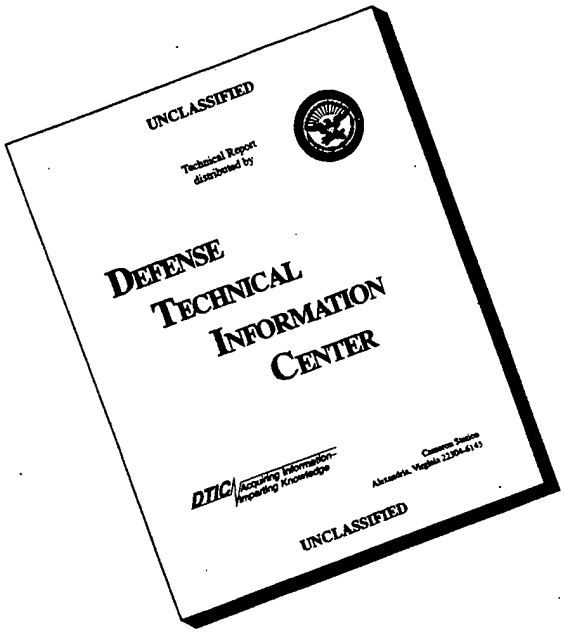

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14. ABSTRACT *(Maximum 200 words)*:

The effects of Bright light (BL) Non-Steriodal Anti-Inflammatory Drugs (NSAIDs) and caffeine on nighttime melatonin, body temperature, alertness, and performance were studied across 48 hours of sleep deprivation. A total of 180 subjects participated (80 male college students in two primary experiments and 100 subjects in secondary experiments). All secondary experiments are presented in the Appendices. Participants in the primary experiments were assigned to either a dim-light (DL) placebo condition, NSAIDs condition-400 mg of ibuprofen, a 200 mg of caffeine condition (twice during the nighttime), a BL condition, or a combined bright and caffeine condition. BL was 2,000 lux or greater; DL was 100 lux or less. The BL condition and the caffeine condition had immediate effect on nighttime melatonin and temperature levels. These latter two conditions decreased melatonin levels and sharply attenuated the normal nocturnal decrease in temperature. However, the combined treatment of BL and caffeine produced the greatest decrease in melatonin and the highest levels of temperature. NSAIDs decreased melatonin relative to placebo, but showed little effect on temperature. Both conditions of BL and caffeine enhanced performance during the nighttime hours. BL tended to enhance performance for tasks without a memory component but had no significant effect on alertness. In contrast, caffeine enhanced alertness and performance for tasks with or without strong memory components. But the combined treatment of BL and caffeine was the most powerful for enhancing all measures.

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FACILITATIVE EFFECTS ON PERFORMANCE FOLLOWING MODIFICATION OF CIRCADIAN RHYTHMS

CONTENTS

Page

INTRODUCTION 1

METHOD 2

Participants 2
Dependent Measures 3
Procedure 3
Design - Experiment 1 4
Design - Experiment 2 4

RESULTS 4

EXPERIMENT 1: BRIGHT LIGHT AND NSAIDs EFFECTS 4

Hypothesis 1: Treatment Effects on Circadian Rhythms (Melatonin and Temperature) 4
Hypothesis 2: Treatment Effects on Behavior (Alertness, Cognition, and Performance) 12

EXPERIMENT 2: BRIGHT LIGHT AND CAFFEINE EFFECTS 16

Hypothesis 3: Treatment Effects on Circadian Rhythms (Melatonin and Temperature) 16
Hypothesis 4: Relationship Between Melatonin and Temperature Levels 18
Hypothesis 5: Treatment Effects on Behavior (Alertness, Cognition, and Performance) 21

SUMMARY 25

Melatonin and Temperature 25
Alertness, Cognition, and Performance 26

DISCUSSION 27

REFERENCES 29

APPENDIX A: BRIGHT LIGHT AFFECTS BODY TEMPERATURE AND MELATONIN LEVELS DURING 48 HOURS OF SLEEP DEPRIVATION A-1

B LIGHT VISOR STUDY: EFFECTS OF ALTERNATING BRIGHT AND DIM LIGHT ON TEMPERATURE B-1

C: RELIABILITY OF SALIVARY MELATONIN C-1

D: NSAID EFFECTS ON BODY TEMPERATURE AND MELATONIN SYNTHESIS D-1

Page

APPENDIX E: METHOD E-1

F: PERFORMANCE TASKS F-1

G: RESULTS - EXPERIMENT 1: BRIGHT LIGHT AND NSAID EFFECTS G-1

H: RESULTS - EXPERIMENT 2: BRIGHT LIGHT AND CAFFEINE EFFECTS H-1

I: PERSONNEL WORKING ON THE PROJECT ENTITLED "FACILITATIVE EFFECTS ON PERFORMANCE FOLLOWING MODIFICATION OF CIRCADIAN RHYTHMS" - CONTRACT # MDA903-93-K-0002 ..... I-1

J: DISSERTATIONS SUPPORTED IN PART BY CONTRACT # MDA903-93-K-0002 ..... J-1

K: ABSTRACTS, PUBLICATIONS PRESENTATIONS SUPPORTED BY CONTRACT # MDA903-93-K-0002 ..... K-1

FIGURE CAPTIONS L-1

LIST OF TABLES

Table 1. Correlations Between Circadian Rhythm Parameters (Amplitude, Phase, and Mesor) for Melatonin Obtained on Night 1 and Those Obtained on Night 2 10

2. Correlations Between Circadian Rhythm Parameters (Amplitude, Phase, and Mesor) for Temperature Obtained on Night 1 and Those Obtained on Night 2 11

3. Percentage of Time Performance was Better Under Treatment Condition for Experiment 1 13

4. Individual Subject Correlations Between Melatonin and Temperature From 2100 to 0800 hr 20

5. Percentage of Time Performance was Better Under Treatment Conditions Compared to Dim Light Placebo for All Measures (e.g., Throughput, Speed, Accuracy, Lapses, Control Losses, Reaction Time, % Correct, % Incorrect) 24

6. List of Tasks and Measures 55

7. Results of the Night x Condition x Time of Night Test Comparing the Melatonin Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Both Nights 63

8. Results of the Condition x Time of Night Test Comparing the Melatonin Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Night 1 64

Page

LIST OF TABLES (Continued)

Table 9. Results of the Condition x Time of Night Test Comparing the Melatonin Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Night 2 64

10. Results of the Condition x Time of Night Test Comparing the Melatonin Data in the BLN1/DLN2 and DLN1/DLN2 Groups on Night 1 64

11. Results of the Night x Condition x Time of Night Test Comparing the Temperature Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Both Nights 65

12. Results of the Condition x Time of Night Test Comparing the Temperature Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Night 1 65

13. Results of the Condition x Time of Night Test Comparing the Temperature Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Night 2 66

14. Results of the Condition x Time of Night Test Comparing the Temperature Data in the BLN1/DLN2 and DLN1/DLN2 Groups on Night 1 67

15. Results of the Night x Condition x Time of Night Test Comparing the Melatonin Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Both Nights 68

16. Results of the Condition x Time of Night Test Comparing the Melatonin Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Night 1 69

17. Results of the Condition x Time of Night Test Comparing the Melatonin Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Night 2 69

18. Results of the Night x Condition x Time of Night Test Comparing the Temperature Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Both Nights 70

19. Results of the Condition x Time of Night Test Comparing the Temperature Data NSN1/NSN2 and DLN1/DLN2 Groups on Night 1 71

20. Results of the Condition x Time of Night Test Comparing the Temperature Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Night 1 71

21. Results of the Condition x Time of Night Test Comparing the Melatonin Difference Scores Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2 74

22. Results of the Condition Test Comparing the Circadian Amplitude of the Melatonin Rhythm Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2 75

Page

LIST OF TABLES (Continued)

Table 23. Results of the Condition Test Comparing the Circadian Phase of the Melatonin Rhythm Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2 76

24. Results of the Condition x Time of Night Test Comparing the Temperature Difference Scores Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2 77

25. Results of the Condition Test Comparing the Circadian Amplitude of the Temperature Rhythm Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2 77

26. Results of the Condition Test Comparing the Circadian Phase of the Temperature Rhythm Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2 78

27. F rations for the Significant Findings Obtained during the Statistical Analysis of Hypothesis 2b 92

28. F ratios for the Significant Findings Obtained during the Statistical Analysis of Hypothesis 2c 97

29. Analysis of Variance Summary Table for Melatonin and Temperature Data on Night 1 and Night 2 97

30. List of Performance Tasks and Measures Analyzed 109

31. Mean percentage of Time Which Performance Was Better Under Treatment Compare to Placebo for Each Condition 110

32a. Analysis of Variance Summary Table for 2 x 2 x 2 (Drug Condition x Light Condition Night) Repeated Measures Analysis. Tasks With a Memory Component 118

32b. Analysis of Variance Summary Table for 2 x 2 x 2 (Drug Condition x Light Condition Night) Repeated Measures Analysis. Tasks Without a Memory Component 118

33a. Means for Performance Data: Effect of Sleep Deprivation (Night 1 vs. Night 2). Tasks With a Memory Component 120

33b. Means for Performance Data: Effect of Sleep Deprivation (Night 1 vs. Night 2). Tasks Without a Memory Component 120

Page

LIST OF TABLES (Continued)

Table 34a. Analysis of Variance Summary Table for 2 x 2 x 4 (Drug Condition x Light Condition x Time-of-Night) Repeated Measures Analysis. Tasks With a Memory Component 122

34b. Analysis of Variance Summary Table for 2 x 2 x 4 (Drug Condition x Light Condition x Time-of-Night) Repeated Measures Analysis. Tasks With a Memory Component 123

34c. Analysis of Variance Summary Table for 2 x 2 x 4 (Drug Condition x Light Condition x Time-of-Night) Repeated Measures Analysis. Tasks Without a Memory Component 124

FACILITATIVE EFFECTS ON PERFORMANCE FOLLOWING MEODIFICATION OF CIRCADIAN RHYTHMS

Introduction

The research presented here focuses on interventions used to reduce degradations in alertness and performance across a 48-hr period of sleep deprivation. Specifically, the paper describes a series of studies dealing with the effects of bright light, nonsteroidal anti-inflammatory drugs (NSAIDs), caffeine and the simultaneous administration of bright light and caffeine on circadian rhythms (melatonin and temperature) and behavior (alertness, cognition, and performance).

Maintaining nighttime alertness and high levels of performance are vital for some occupations, and especially so for individuals working out of phase with their circadian rhythms; i.e., working when they normally would be sleeping. The latter includes military operations personnel during sustained or continuous operations and shift workers in general. During the early morning hours (0200-0600 hr), performance and alertness are at their lowest level (Colquhoun, 1981; Smith, 1992). The trough in alertness and performance is negatively related to the circadian rhythm of the hormone melatonin and positively related to the rhythm of body temperature. When melatonin is high and body temperature low, alertness and performance are at their minimum levels. Treatments (e.g., exposure to bright light) which decrease melatonin and attenuate the nighttime decrease in temperature improve alertness and performance (e.g., Badia, 1991). The treatments used in the current report were selected on the basis of both experimental evidence and theoretical considerations relating to the effects that body temperature and nighttime melatonin levels have on alertness, cognition, and performance. Exposure to nighttime bright light is known to suppress melatonin production and attenuate the drop in body temperature. Based upon theoretical considerations, we hypothesized that ingestion of NSAIDs or caffeine would have similar effects.

The results are reported in two parts (Experiments 1 and 2). The first part focuses on the effects of nighttime bright light exposure and the ingestion of NSAIDs on alertness, performance, melatonin and temperature during 48-hrs of sleep deprivation. The second part of the report focuses on the effects that nighttime bright light exposure and the ingestion of caffeine have on the same measures under the same conditions.

Method

Participants

Research participants (N = 180) were male graduate and under-graduate students between the ages of 18 and 28, enrolled at Bowling Green State University. A total of 80 individuals participated in Experiments 1 and 2 of this report. The remaining subjects participated in pilot work presented in Appendix A, B, C and D.

Subjects were rigorously screened prior to participation in the study. Only subjects free of significant medical and psychological conditions, having a regular sleep/wake cycle, not using nicotine or medications but regular users of caffeine (50-200 mg per day) were tested. In addition, subjects were restricted from drug use prior to participation (including caffeine, alcohol and non-prescribed drugs). Subjects were required to complete several forms prior to participation. After giving informed consent, subjects provided their medical history, rest-activity schedule, food and chemical sensitivities and demographics. Subjects were paid $120 for completing the study. Those subjects who retired from the study early were paid on a prorated basis. See Appendix E for further details of subject characteristics and method.

Dependent Measures

Salivary melatonin was sampled every 60 min throughout Experiment 1 and every 60 min during the nighttime hours (2000-0800 hr) during Experiment 2. Melatonin content in saliva was measured by radio immunoassay (Elias, usa, Osceola, WI. USA). Tympanic temperature ("First Temp" - Intelligent Medical Systems, Carlsbad, CA, USA) was recorded in °C every 60 min. Alertness and performance were assessed every 3 hr during the nighttime (i.e., 2100-0900 hr for Experiment 1 and 2000-0800 hr for Experiment 2). Alertness measures included the Maintenance of Wakefulness Test (Miltner et al., 1982), Stanford Sleepiness Scale (Hoddes et al., 1973), and spectral analysis of the EEG. Performance was assessed using tasks from the Walter Reed Assessment Battery (Thorne et al., 1985), the United Tri-service Performance Assessment Battery (UTCPAB - Thorne et al., 1990) as well as tasks developed by our lab and others (e.g., Dinges, et al., 1993, 1994). For a complete listing of performance tasks used see Appendix F.

Procedure

The testing session began at 2030 hr Thursday and continued until 1030 hr Sunday. After sleeping from 0030-1030 hr on Thursday night, the participants remained awake for 48 hr. During this 48-hr period, a constant routine was enforced with no time cues being provided to the participants. Subjects remained seated throughout the experiment. Exposure to nighttime bright light (≥ 2,500 lux) occurred from 2100 to 0900 hr (Experiment 1) and from 2000 to 0800 hr (Experiment 2). Otherwise, subjects were exposed to dim light (≤ 100 lux). Administration of NSAIDs, caffeine, or placebo was double blind and occurred at 2100 and 0300 hr (Experiment 1) and 2000 and 0200 hr (Experiment 2) each night.

Design - Experiment 1

A mixed design utilizing four different combinations of bright light (>2,500 lux), dim light (<100 lux), NSAID (400 mg ibuprofen), and placebo (400 mg sugar) was used. The combinations were: (a) bright light on Night 1 (2100-0900 hr) followed by bright light on Night 2 (2100-0900 hr) (BLN1/BLN2; n=10); (b) dim light on Night 1 followed by dim light on Night 2 (DLN1/DLN2; n=10); (c) bright light on Night 1 (2100-0900 hr) followed by dim light on Night 2 (BLN1/DLN2; n=10); and (d) NSAID on Night 1 followed by NSAID on Night 2 (NSN1/NSN2; n=10).

Design - Experiment 2

A mixed design utilizing four treatment conditions: dim-light placebo (<100 lux plus placebo (200 mg sugar); n=10), bright-light plus placebo (> 2,500 lux/200 mg sugar; n=10), dim- light plus caffeine (<100 lux/200 mg caffeine; n=10), and bright-light plus caffeine (> 2,500 lux/200 mg caffeine; n=10) was used.

Results

Experiment 1: Bright Light and NSAIDs Effects

We present first the effects of bright light and NSAIDs administration on the physiological measures of melatonin and temperature. This section is followed by a section describing the effects of these treatments on the behavioral measures of alertness, cognition, and performance.

Hypothesis 1: Treatment Effects on Circadian Rhythms (Melatonin and Temperature)

Hypothesis 1a

Hypothesis. Exposure to bright light stimulation during the nocturnal hours of Nights 1 and 2 will suppress melatonin and will attenuate the decrease in Nighttime temperature relative to

exposure to dim light stimulation. A related hypothesis was that these effects will occur in a similar manner during both Nights 1 and 2.

Rationale. There is considerable literature indicating that bright light suppresses melatonin (Lewy et al., 1980; Myers et al., 1994) and enhances temperature (Badia et al., 1991; Myers et al., 1994). Nighttime bright light exposure is thought to suppress melatonin by preventing noradrenergic stimulation of beta-adrenergic receptors on the pineal gland (Reiter 1991). It is unknown if such light-induced effects can be obtained (a) throughout the nighttime hours and (b) across consecutive 24-hr periods. Since it is known that melatonin can be suppressed in nonhumans throughout the nocturnal period and across several cycles (Phillips & Berger, 1992) and since the circadian system is similar across species (Wurtman et al., 1968), effects similar to those obtained in nonhumans were predicted. Preliminary results from research funded by this grant showed that melatonin and temperature levels can be controlled with the use of nighttime photic stimulation (Appendix A & B). In the latter studies, subjects were exposed to 60 or 90 min alternating blocks of bright and dim light using either stationary light sources or light visors (which allow subjects mobility during photic stimulation). Results using the stationary light boxes indicated that while under bright light, melatonin levels decreased and temperature levels increased, whereas under dim light, melatonin levels increased and temperature decreased. Results using the light visors were inconclusive (Appendix B).

Results. Visual analysis of the data indicate that bright light suppressed melatonin and enhanced temperature relative to dim light (Figures 1-4). Similar results were obtained on both Night 1 and Night 2. Statistical analyses supported these observations. In each bright light-dim light comparison (N1: BLN1/BLN2-DLN1/DLN2; N1: BLN1/DLN2-DLN1/DLN2; N2: BLN1/BLN2-DLN1/DLN2), either a significant Condition x Time interaction or a significant

main effect of Condition was obtained. These findings were obtained for both melatonin and temperature (see Appendix G for full treatment of statistical results).

Conclusion. Bright light stimulation on Nights 1 and 2 suppressed melatonin and enhanced temperature throughout the nighttime hours. No effect of Night was obtained indicating that nocturnal exposure to bright light was equally effective for both Night 1 and Night 2.

Hypothesis 1b

Hypothesis. NSAID administration on Nights 1 and 2 will suppress melatonin and attenuate the nighttime decrease in temperature relative to placebo administration. A related hypothesis was that these effects will occur in a similar manner across both nights.

Rationale. A few studies, including preliminary work funded by this grant have shown that NSAID administration suppresses melatonin (Murphy et al., 1993, 1994, in press; Surrall et al., 1988) and enhances temperature (Murphy et al., 1993, 1994; Appendix D). These experiments indicate that the duration of such effects are related to the half life (typically 6 hr) of the NSAID tested (Surrall et al., 1988). However, it is unknown if such NSAID-induced effects can be obtained (a) throughout the nighttime hours and (b) across consecutive 24-hr periods. The latter effects appear likely since NSAIDs and bright light appear to have similar effects on melatonin/temperature and may achieve these effects through similar mechanisms. They also appear likely since bright light can affect the two measures throughout the nocturnal period and across several cycles in nonhumans.

Results. Visual analysis of the data suggest differential melatonin and temperature results: Relative to a placebo condition, NSAIDs suppressed melatonin but had little effect on temperature. Similar results were obtained on both Nights 1 and 2(Figures 5-6). Statistical analyses supported these observations. A significant main effect of Condition (NSN1/NSN2-

DLN1/DLN2) was found for melatonin on both nights. No significant effects were found for temperature on either night. In addition, the melatonin and temperature findings were very similar on both nights and no significant effect of Night was obtained (see Appendix G for full treatment of statistical results).

Conclusion. The administration of NSAIDs was equally effective in suppressing melatonin on both nights. However, NSAIDs had little effect on temperature during either night. Given the "tight fit" between melatonin and temperature, it is unclear why NSAIDs affected melatonin but not temperature.

Hypothesis 1c

Hypothesis. Bright light stimulation on Night 1 will reduce the circadian amplitude for melatonin and temperature during dim light stimulation on Night 2 relative to subjects receiving dim light stimulation on both nights. Two related hypotheses were also tested (a) bright light stimulation on Night 1 will reduce the circadian amplitude of melatonin and temperature during dim light stimulation on Night 2 to an extent similar to that induced in subjects receiving bright light stimulation on both nights and, (b) bright light stimulation on Night 1 will not affect the circadian phase of melatonin and temperature during dim light stimulation on Night 2 relative to subjects receiving either dim or bright light stimulation on both nights.

Rationale. Photic stimulation affects circadian amplitude with diurnal light increasing amplitude and nocturnal light decreasing amplitude (Czeisler et al., 1992). Photic stimulation also affects circadian phase with evening light delaying phase and morning light advancing phase (Czeisler et al., 1989). Nighttime exposure to bright light will have immediate effects on Night 1 suppressing melatonin and temperature. In addition, bright light exposure on Night 1 will also have delayed circadian effects observed under dim light exposure on Night 2. Specifically, a

reduction in melatonin and temperature amplitude was predicted for both Night 1 and 2. Since both evening and morning light was used, no change in phase was predicted; i.e. phase change should cancel out.

Results. Visual analysis of the melatonin and temperature data reveal that exposure to bright light on Night 1 reduced amplitude during exposure to dim light on Night 2 relative to exposure to dim light on both nights (Figures 7 and 8). This reduction was similar to that obtained under bright light on Night 2 following bright light on Night 1. Some of the statistical analyses supported this observation while others did not (see Appendix G for full treatment of statistical results). Evaluation of the difference score data for Night 2 indicated that for melatonin the BLN1/DLN2 group was not significantly different from the DLN1/DLN2 group, but was different from the BLN1/BLN2 group and that for temperature the BLN1/DLN2 group was significantly different from the DLN1/DLN2 group but not from the BLN1/BLN2 group. Evaluation of the melatonin and temperature data for delayed circadian effects showed neither amplitude nor phase were changed under dim light on Night 2 following bright light on Night 1.

Conclusion. Bright light stimulation on Night 1 reduces circadian amplitude during dim light stimulation on Night 2 to an extent similar to that induced by bright light stimulation on both nights. These results confirm that exposure to nighttime bright light has immediate effects and also delayed circadian effects which carryover to Night 2. Bright light stimulation during the evening and early morning hours on Night 1 does not alter circadian phase on Night 2 (i.e., does not result in a phase advance or phase delay of the temperature rhythm).

Hypothesis 1d

Hypothesis. Circadian rhythms in melatonin and temperature measured under constant dim light (in the absence of masking factors) will be reliable (i.e., highly correlated) between Night 1 and Night 2.

Rationale. Melatonin and temperature are common measures of circadian rhythmicity and these measures are considered stable within an individual across multiple nights. However, this reliability has been demonstrated in only a few studies: Only two studies measuring the melatonin rhythm (Arato et al., 1985; Claustrat et al., 1986) and only two studies measuring the temperature rhythm (Aschoff & Wever, 1980; Kleitman & Ramsaroop, 1948) presented convincing findings. Because the present study measured circadian rhythms under constant conditions, it was predicted that melatonin and temperature would be reliable across Night 1 and 2. Preliminary work suggested that salivary melatonin was reliable during short term periods of sleep deprivation (see Appendix C).

Results. Tables 1-2 present the average circadian rhythm scores for the melatonin and temperature data on Nights 1 and 2 in the DLN1/DLN2 condition. The correlation between the scores obtained on Night 1 and Night 2 are also shown in the Tables. High, positive, and significant correlations were obtained for all of the circadian measures for melatonin and for two of three circadian measures for temperature. The amplitude measure for temperature was not significantly correlated on Nights 1 and 2 due to a few subjects with low correlations.

Conclusion. As hypothesized, circadian rhythms in melatonin and temperature measured under constant dim light (in the absence of masking factors) were reliable (i.e., highly correlated) between Night 1 and Night 2.

Table 1

Correlations Between Circadian Rhythm Parameters (Amplitude, Phase, and Mesor) for Melatonin Obtained on Night 1 and Those Obtained on Night 2

MELATONIN: AMPLITUDE

mean (standard error)
N1: 15.61 (2.70) pg/mL
N2: 20.97 (5.99) pg/mL

correlation coefficient
N1-N2: +.67 ($p < .05$)

MELATONIN: PHASE

mean (standard error)
N1: 0534 (0.47) hr
N2: 0518 (0.55) hr

correlation coefficient
N1-N2: +.95 ($p < .05$)

MELATONIN: MESOR

mean (standard error)
N1: 12.25 (2.34) pg/mL
N2: 13.23 (4.14) pg/mL

correlation coefficient
N1-N2: +.91 ($p < .05$)

Table 2

Correlations Between Circadian Rhythm Parameters (Amplitude, Phase, and Mesor) for Temperature Obtained on Night 1 and Those Obtained on Night 2.

TEMPERATURE: AMPLITUDE

mean (standard error)
N1: 0.27 (0.03) °C
N2: 0.30 (0.02) °C

correlation coefficient
N1-N2: -.11 ($p > .05$)

TEMPERATURE: PHASE

mean (standard error)
N1: 0545 (0.72) hr
N2: 0530 (0.28) hr

correlation coefficient
N1-N2: +.81 ($p < .05$)

TEMPERATURE: MESOR

mean (standard error)
N1: 37.12 (0.08) °C
N2: 37.14 (0.06) °C

correlation coefficient
N1-N2: +.94 ($p < .05$)

Hypothesis 2: Treatment Effects on Behavior (Alertness, Cognition, and Performance)

Hypothesis 2a

Hypothesis. Exposure to bright light stimulation on Nights 1 and 2 will enhance alertness and performance relative to exposure to dim light stimulation. A related hypothesis was that these effects will occur in a similar manner across consecutive 24-hr periods.

Rationale. During the nighttime, melatonin increases and temperature decreases. These changes are associated with decreases in alertness and performance. Bright light suppresses melatonin and enhances temperature and should therefore increase alertness and performance. These effects of photic stimulation have been demonstrated (Badia et al., 1991); however, it is unknown if such light-induced effects can be obtained (a) throughout the nighttime hours and (b) across consecutive 24-hr periods.

Results. Most alertness and performance measures exhibited the expected Time-of-Night (worse between 0200-0600 h each night) and Night effects (worse performance on Night 2 compared to Night 1 of sleep deprivation). Bright light was able to attenuate these changes on some measures (Table 3). For example, bright light significantly decreased relative EEG power in the theta and alpha bands, thus indicating higher arousal, and bright light also increased performance on the dual task (control losses) and continuous recognition task (throughput) relative to dim light (see Appendix G for full treatment of statistical results).

Conclusion. Exposure to bright light stimulation on Nights 1 and 2, in general, resulted in enhanced alertness levels and performance on several measures.

Table 3

Percentage of Time Performance was Better Under Treatment Condition for Experiment 1

	Bright Light	NSAIDS
Dual Task		
Throughput	43	29
Control Losses	86	86
RMS	100	100
Probed Memory Recall		
# Correct	33	25
Continuous Recognition Task		
Accuracy	13	63
Throughput	100	75
Reaction Time	75	75
Switching Task - Mannequin		
Throughput	13	25
Reaction Time	88	100
Switching Task - Math Processing		
Throughput	50	63
Reaction Time	88	63
Procedural Memory - Basic		
Throughput	25	12
Reaction Time	0	12
Procedural Memory - Coded		
Throughput	50	0
Reaction Time	25	12
Stanford Sleepiness Scale	100	88
EEG Theta	88	50
Maintenance of Wakefulness Test	50	13

Hypothesis 2b

Hypothesis. Nonsteroidal anti-inflammatory drug administration on Nights 1 and 2 will enhance performance and alertness relative to placebo administration. A related hypothesis was that these effects will occur in a similar manner across consecutive 24-hr periods.

Rationale. During the nighttime, melatonin increases and temperature decreases. These changes are associated with decreases in alertness and performance. Nonsteroidal anti-inflammatory drugs suppress melatonin and enhance temperature and should therefore increase alertness and performance. Only the effects of NSAIDs on melatonin and temperature have been shown previously (Murphy et al., 1993, 1994, in press). Further, it is unknown if such NSAID-induced effects can be obtained (a) throughout the nighttime hours and (b) across consecutive 24-hr periods.

Results. All alertness and most performance measures exhibited the expected Time-of-Night (worse between 0200-0600 h each night) and Night effects (worse performance on Night 2 compared to Night 1 of sleep deprivation). While NSAID administration appeared to enhance alertness and performance on some tasks (Table 3), no statistically significant effects were found (see Appendix G for full treatment of statistical results).

Conclusion. Contrary to what was hypothesized, nonsteroidal anti-inflammatory drugs did not have a significant effect on alertness and performance during sleep deprivation.

Hypothesis 2c

Hypothesis. Exposure to bright light stimulation on Night 1 will enhance alertness and performance during subsequent dim light stimulation on Night 2 relative to exposure to dim light stimulation on both nights: i.e. for subjects receiving bright light on Night 1 and dim light on Night 2, there will be a delayed circadian effect of bright light on alertness and performance that

will carryover to Night 2. That is, subjects should perform on Night 2 under exposure to dim light in a similar manner to those receiving bright light on Night 2.

Rationale. Photic stimulation affects circadian amplitude in that exposure to bright diurnal light results in an increase in amplitude and exposure to bright nocturnal light results in a decrease in amplitude (Czeisler et al., 1992). Alertness and performance are closely related to the amplitude of the melatonin (i.e., the peak) and temperature (i.e., the trough) rhythms (Akerstedt, et al., 1982; Badia et al., 1991). High melatonin and low temperature levels are associated with greater sleepiness and poor performance. If the amplitude of the melatonin and temperature rhythms on Night 2 is reduced by nocturnal exposure to bright light on Night 1 as expected, then alertness and performance during the nighttime hours on Night 2 should be enhanced compared to subjects receiving dim light for both Nights 1 and 2.

Results. Most alertness and performance measures exhibited the expected Time-of-Night (worse between 0200-0600 h each night) and Night effects (worse performance on Night 2 compared to Night 1 of sleep deprivation). Bright light on Night 1 had positive effects on one measure of alertness during dim light on Night 2. Specifically, theta power was significantly lower in the bright light Night 1-dim light Night 2 group compared to the dim light Night 1-dim light Night 2 group on Night 2 (see Appendix G for full treatment of statistical results). Bright light on Night 1 had little positive effect on performance during dim light on Night 2.

Conclusion. Nighttime exposure to bright light during Night 1 appears to have little carryover effect on alertness and performance during dim light stimulation on Night 2. To enhance alertness and performance, bright light stimulation is necessary.

Results

Experiment 2: Bright Light and Caffeine Effects

We present next the effects of bright light and caffeine administration on the physiological measures of melatonin and temperature. The latter physiological section is then followed by a section describing the effects of bright light and caffeine treatments on the behavioral measures of alertness, cognition, and performance.

Hypothesis 3: Treatment Effects on Circadian Rhythms (Melatonin and Temperature).

The following analyses address whether or not the caffeine and light treatments affected nighttime melatonin and temperature levels during two consecutive nights of sleep deprivation.

Hypothesis. Either bright light stimulation alone or caffeine ingestion alone will reduce nighttime melatonin levels and attenuate the nocturnal decrease in temperature relative to the dim light-placebo condition. Moreover, the combined treatment of bright light and caffeine will affect melatonin and temperature levels the most: that is, melatonin levels will be lowest and temperature levels highest in the combined treatment condition. These effects of the treatments on melatonin and temperature levels will be similar for both Night 1 and Night 2.

Rationale. As noted, bright light stimulation during the nighttime suppresses melatonin and enhances temperature relative to dim light stimulation. Bright light is thought to suppress melatonin by preventing noradrenergic stimulation of beta-adrenergic receptors on the pineal (Reiter, 1991). The ability of bright light to attenuate the nocturnal drop in temperature is thought to be related to the suppression of melatonin (Badia et al., 1993). Results from Experiment 1 showed that bright light was able to suppress melatonin and enhance temperature across consecutive 24-hr periods. Thus, a replication of these latter results were predicted.

The effects of caffeine on melatonin synthesis are currently unknown. However, since adenosine is involved in melatonin synthesis (e.g., Gharib et al., 1989), and since caffeine blocks the effects of adenosine (e.g., Daly, 1993; Daly et al., 1981), it was expected that caffeine would decrease nocturnal melatonin levels. Through the blockade of adenosine in the peripheral and central nervous system, caffeine was also expected to attenuate the nocturnal decrease in temperature (e.g., Daly, 1993; Daly et al., 1981). The effects of caffeine on melatonin and temperature were predicted to be similar for both nights.

Since caffeine and bright light may affect melatonin and temperature by different mechanisms (i.e., caffeine by blocking adenosinergic and bright light by blocking beta-adrenergic receptor stimulation), it is expected that the combined treatment of caffeine and bright light will be more effective in suppressing melatonin and enhancing temperature compared to either treatment alone. In addition to possible additive effects due to the multiple mechanisms, caffeine may also directly enhance the ability of bright light to suppress melatonin. Specifically, the amount of light hitting the retina may be increased by caffeine through pupillary dilation (Lin et al., 1994) which may potentiate the effect of light on the SCN (i.e., a lower level of light with the addition of caffeine, may suppress melatonin the same amount that a higher light level would without caffeine).

Results. Both caffeine alone and bright light alone significantly reduced melatonin and maintained temperature at higher levels when compared to dim light placebo. As predicted, the combined bright-light caffeine condition exhibited the most marked effects on melatonin and temperature levels. Melatonin levels were lowest and temperature levels were highest in the combined bright light-caffeine condition (Figures 9-10). Melatonin data were similar for both nights of sleep deprivation within each treatment condition. In contrast, Night 1-Night 2

differences appeared to occur for temperature data. Tympanic temperature was higher for the dim-light placebo condition on Night 2 compared to Night 1, but not significantly so. A trend for significantly higher temperature for the bright light alone condition on Night 2 compared to Night 1 was observed. The dim-light caffeine condition showed higher temperature levels on Night 1 compared to Night 2, but not significantly so. The combined caffeine and bright light treatment condition enhanced temperature levels to a similar degree on both nights as predicted (see Appendix H for full treatment of statistical results).

Hourly temperature data showed the typical circadian rhythm for the dim light placebo and bright light placebo conditions (Figure 11). Administration of caffeine alone appeared to decrease the amplitude of the temperature rhythm especially on Night 1. The most impressive finding however, is for the combined bright light caffeine condition. As seen in Figure 11, the amplitude of the rhythm was considerably reduced and the overall rhythm flattened. For this condition, temperature was maintained at high levels throughout the entire deprivation period.

Conclusions. Both caffeine alone and bright light alone were effective treatments for reducing melatonin levels and attenuating the nightly decrease in temperature during sleep deprivation. The effects of caffeine on temperature were not as pronounced on Night 2 compared to Night 1. The most efficacious treatment for decreasing melatonin and attenuating the decrease in nighttime temperature is the combination of bright-light and caffeine.

Hypothesis 4: Relationshipbetween Melatonin and Temperature Levels

The following analyses address the question of whether the different treatments affected the relationship between melatonin and temperature.

Hypothesis. There will be a negative correlation between melatonin and temperature levels. Since the caffeine and bright light treatments are expected to reduce the amount of endogenous

melatonin and attenuate the decrease in body temperature. the treatments may effect the relationship between the two measures. Specifically. since the treatments are expected to mask the endogenous rhythms and reduce the range of scores for the melatonin and temperature data . the treatments are expected to reduce the correlation between the two measures.

Rational. Melatonin levels will be negatively correlated with temperature levels (Badia et al., 1993). That is. subjects with higher levels of endogenous melatonin will show larger decreases in temperature. This effect will occur irrespective of condition; but. it will be especially evident in the continuous dim-light placebo condition since (a) the masking effects of photic stimulation and of caffeine intake will not be present and (b) the range of values will not be restricted.

Results. Almost all subjects in the dim-light placebo. bright-light placebo and dim-light caffeine groups showed a significant negative correlation between melatonin and temperature (Table 4 -transformed data). On the other hand. less than half of the individuals in the combined treatment group showed a significant negative correlation between melatonin and temperature levels. Averaging across individual subjects for the transformed data, yields the highest average correlation for the dim-light placebo condition. The average correlation between melatonin and temperature was significantly lower for the bright-light placebo condition relative to the dim-light placebo condition on Night 1. The combined treatment of caffeine and bright light showed a significantly smaller correlation between melatonin and temperature compared to the dim-light placebo condition on both nights of sleep deprivation.

Lastly, a correlation between the average curve of melatonin and temperature for each condition was calculated (Figure 12). As evident in the Figure. the negative correlation between melatonin and temperature was high for all conditions. albeit the correlation for the caffeine conditions were lower (See Appendix H for full treatment of statistical results).

Table 4

Individual Subject Correlations* Between Melatonin and Temperature From 2100 to 0800 hr

Subject Number	Condition	Night 1 Raw r	Night 1 Trans r	Night 2 Raw r	Night 2 Trans r
301	DLP	-.47 ns	-.53 (.08)	-.62	-.87
310	DLP	-.42 ns	-.56 (.06)	-.82	-.94
311	DLP	-.86	-.90	-.79	-.85
313	DLP	-.81	-.86	-.81	-.79
320	DLP	-.77	-.83	-.78	-.88
325	DLP	-.94	-.95	-.93	-.92
328	DLP	-.64	-.68	-.60	-.68
334	DLP	-.74	-.72	-.77	-.80
343	DLP	-.70	-.76	-.92	-.90
344	DLP	-.76	-.81	-.86	-.82
Average r	***DLP***	***-.71***	***-.76***	***-.79***	***-.85***
302	DLC	-.59	-.57	-.47 ns	-.59
303	DLC	-.36 ns	-.64	-.51(.09)	-.63
307	DLC	-.54(.07)	-.56 (.06)	-.89	-.89
319	DLC	-.85	-.83	-.90	-.84
326	DLC	-.53(.08)	-.56 (.06)	-.86	-.77
327	DLC	-.67	-.65	-.80	-.82
333	DLC	-.70	-.72	-.75	-.71
336	DLC	-.51(.09)	-.65	.03 ns	-.08 ns
350	DLC	-.74	-.65	-.93	-.96
Average r	***DLC***	***-.61***	***-.65***	***-.68***	***-.70***
305	BLP	-.47 ns	-.76	-.68	-.73
308	BLP	-.52(.08)	-.74	-.92	-.93
321	BLP	-.74	-.72	-.88	-.94
324	BLP	-.90	-.87	-.60	-.71
330	BLP	.09 ns	-.09 ns	.04 ns	-.04 ns
337	BLP	-.68	-.78	-.72	-.80
339	BLP	-.66	-.66	-.62	-.67
342	BLP	-.63	-.72	-.75	-.63
346	BLP	-.94	-.95	-.93	-.95
348	BLP	-.56(.06)	-.72	-.56(.06)	-.60
Average r	***BLP***	***-.60***	***-.70***	***-.66***	***-.70***
306	BLC	-.01 ns	-.01 ns	-.20 ns	-.32 ns
312	BLC	-.41 ns	-.40 ns	-.85	-.86
323	BLC	-.56(.06)	-.56 (.06)	-.35 ns	-.43 ns
331	BLC	-.70	-.63	-.35 ns	-.34 ns
332	BLC	-.87	-.80	-.91	-.88
338	BLC	-.11 ns	-.24 ns	-.86	-.89
340	BLC	-.67	-.74	-.79	-.71
341	BLC	.16 ns	.20 ns	-.59	-.63
345	BLC	-.36 ns	-.43 ns	-.52(.09)	-.49 ns
347	BLC	-.25 ns	-.26 ns	.25 ns	.40 ns
Average r	***BLC***	***-.38***	***-.39***	***-.52***	***-.52***

Note. All correlation's are significant unless otherwise noted ($p<.05$). ns = non-significant. Values in parentheses represent p values for trends.

DLP = dim light placebo. DLC = dim light caffeine. BLP = bright light placebo. BLC = bright light caffeine. Raw = Raw Data. Trans = Transformed Data

Conclusion. A significant negative correlation exists for melatonin and temperature. The negative relationship between melatonin and temperature is observed most clearly under continuous dim light in the absence of masking factors. The presence of masking factors imposed by the treatments tended to reduce the relationship between melatonin and temperature.

Hypothesis 5: Treatment Effects on Behavior (Alertness, Cognition, and Performance).

The following analyses address the question of how well the caffeine and light treatments maintained alertness and performance across the two nights of sleep deprivation.

Hypothesis. Both caffeine ingestion alone and bright light exposure alone will lead to enhanced alertness and performance during the nighttime hours compared to dim light placebo. Caffeine administration will be more effective in enhancing nighttime alertness and performance than exposure to bright photic stimulation. However, the combined treatment of bright light and caffeine is expected to be most effective in enhancing alertness and performance. Both alertness and performance are expected to be worse on Night 2 compare to Night 1.

Rationale. Previous research has shown both caffeine ingestion alone and bright light exposure alone to enhance alertness and performance during sleep deprivation (e.g., Badia et al., 1991; Bonnet & Arand, 1994a, 1994b). Bright light influences alertness and performance through suppression of melatonin, attenuation of the nocturnal decrease in temperature and increases in cortical arousal (e.g., Badia et al., 1991; Campbell & Dawson, 1990; Daurat et al., 1993; Dollins, Lynch, Wurtman, Deng & Lieberman, 1993).

Caffeine, in its excitatory action, affects many processes which influence alertness and performance (e.g., caffeine: decreases muscle fatigue, increases neurotransmitter release, causes hyperthermia, increases behavioral activity, reverses the hypnogenic effects of adenosine--Daly, 1993; Daly et al., 1981). Since caffeine affects more physiological processes than bright light, it

was hypothesized that caffeine ingestion would be more effective in enhancing nighttime alertness and performance compared to exposure to bright light.

The combined treatment of bright light and caffeine was expected to be more effective in enhancing alertness and performance compared to either treatment alone. As noted, caffeine alone and bright light alone affect alertness and performance in different ways. The combined treatment of bright light and caffeine may thus lead to larger positive effects on nighttime alertness and performance.

Results - Alertness Measures

Maintenance of Wakefulness Test. Both caffeine alone and the combined treatment of bright light and caffeine produced significantly longer latencies to sleep on the Maintenance of Wakefulness Test (MWT) compared to dim-light placebo and bright light alone (Figure 13). Alertness on the MWT was enhanced on both nights, especially in the early morning hours, by the caffeine treatment and the combined treatment of bright light exposure and caffeine ingestion. The alerting effects of caffeine also appeared to carry over into the daytime hours. That is, alertness on the daytime MWT was significantly higher in the caffeine treatments compared to no caffeine, even though the test was done 6 ½ to 8 hr after the 0200 h dose of caffeine. In contrast, to our previous findings, bright light alone had little effect on the ability to maintain wakefulness on the MWT during the day or night. Significantly shorter sleep latencies were observed for all conditions on Night 2 compared to Night 1. In addition, alertness was lowest in the early morning hours (see Appendix H for full treatment of statistical results).

Subjective sleepiness (Stanford Sleepiness Scale). Compared to subjects in the dim-light placebo condition, subjects in the combined treatment condition reported feeling significantly more alert on both nights whereas subjects in the caffeine alone (Night 1) and bright light alone

(Night 2) conditions reported feeling significantly more alert for only one night (Figure 14). Subjective sleepiness was significantly higher for all conditions on Night 2 compared to Night 1. In addition, sleepiness was highest in the early morning hours.

Power spectral analysis of the EEG. Little systematic effect of the treatments on EEG data were observed. Data are presented in Appendix H.

Conclusion. Caffeine alone and the combined treatment of bright light and caffeine improved objective alertness (MWT) to a similar degree. Subjects were able to maintain wakefulness much better under the latter conditions. Subjective alertness (SSS) was higher in the combined treatment condition compared to all other conditions. Little positive effect of bright light on objective alertness was observed; however, some improvement in subjective alertness was observed on Night 2.

Results - Performance Measures

In general, the caffeine and bright light treatments showed the largest effects on performance between 2300 and 0800 h each night (noted on Figures 15-18 as measurements at 0030, 0330 and 0630 h). In addition, performance on several tasks was significantly worse on Night 2 compared to performance on Night 1. There was however one task which showed significantly improved performance on Night 2 - Switching Task Mannequin Throughput performance.

The combined bright light caffeine condition, as well as the caffeine alone condition showed better performance compared to dim light placebo on both nights of sleep deprivation. Bright-light alone on the other hand, tended to enhance overall performance relative to dim light placebo on Night 2 with smaller effects on Night 1 (Table 5). In addition, bright light tended to enhance performance on tasks without a memory component whereas the caffeine treatments significantly enhanced performance in both tasks with and without a memory component. A comparison of

Table 5

Percentage of Time Performance Was Better Under Treatment Conditions Compared to Dim Light Placebo for All Measures (e.g., Throughput, Speed, Accuracy, Lapses, Control Losses, Reaction time, %Correct, %Incorrect)

Night 1		Night 2	
Bright-light caffeine	78%	Bright-light Caffeine	90%
Dim-light caffeine	68%	Dim-light Caffeine	70%
Bright-light placebo	52%	Bright-light Placebo	70%

the treatments showed that caffeine alone produced significantly better performance than bright light alone for several measures whereas bright light alone produced significantly better performance than caffeine alone only once (Figures 15-18). Consistent with the melatonin and temperature results, the combined treatment of bright light and caffeine produced the best overall performance. The figures show performance under the combined treatment condition to be significantly better than performance under dim light placebo most of the time and significantly better than performance under bright light alone and caffeine alone for more than one third of the time (see Appendix H for full treatment of statistical results).

Conclusion. Both caffeine alone and bright light alone were effective treatments for enhancing performance during sleep deprivation. Caffeine alone enhanced performance for tasks both with and without a memory component whereas bright light tended to enhance performance for tasks without a memory component. The most effective treatment for enhancing performance is the combined treatment of bright light and caffeine. Therefore, the combination of bright light and caffeine is a powerful and readily available method of enhancing all types of performance during the nighttime hours.

Summary

The above research tested the effects of bright light, caffeine and NSAIDS on circadian rhythms (melatonin and temperature), and behavior (alertness, cognition and performance) during two nights of sleep deprivation. The results are summarized in the following conclusions.

Melatonin and Temperature

1. Both caffeine alone and bright light alone were effective treatments for reducing nighttime melatonin levels and for attenuating the nightly decrease in temperature across two consecutive nights of sleep deprivation. The effects of caffeine on temperature were not as pronounced on Night 2 compared to Night 1. The most efficacious treatment for decreasing melatonin and attenuating the decrease in nighttime temperature for both Night 1 and 2 is the combination of bright-light and caffeine.

2. The administration of NSAIDs was effective in suppressing melatonin on both Night 1 and Night 2. However, NSAIDs had little effect on attenuating the nighttime decrease in temperature on either Night 1 or 2.

3. Bright light stimulation on Night 1 reduces circadian amplitude during dim light stimulation on Night 2 to an extent similar to that induced by bright light stimulation on both nights. Bright light stimulation on Night 1 does not alter circadian phase on Night 2.

4. The circadian rhythms of melatonin and temperature obtained under constant dim light conditions were reliable from night to night.

5. Nighttime melatonin levels and temperature levels are significantly correlated. The negative relationship between melatonin and temperature was observed most clearly under continuous dim light in the absence of treatments. The treatments (bright light, caffeine) tended to reduce the relationship between melatonin and temperature.

Alertness, Cognition and Performance

6. Caffeine alone enhanced objective and subjective measures of alertness. Little effect of bright light on objective alertness was observed; however, some effect on subjective alertness was observed. The combined treatment of bright-light and caffeine was the most effective treatment for enhancing all measures of alertness.

7. Both caffeine alone and bright light alone were effective treatments for enhancing performance during sleep deprivation. In general, when the treatments attenuated the nocturnal decrease in temperature, enhancements in performance were obtained.

8. Continuous bright light stimulation appears necessary for enhancement in performance to occur.

9. Caffeine alone enhanced performance for tasks both with and without a memory component. Bright light tended to enhance performance for tasks without a memory component. The combined treatment of bright-light and caffeine was the most powerful treatment for enhancing all measures of performance during the nighttime hours.

Discussion

Both caffeine and bright light were effective treatments for suppressing melatonin, for attenuating the nocturnal decrease in temperature and for enhancing alertness and performance. However, differential effects of the treatments on alertness and performance were observed. Caffeine administration appears to be a more powerful treatment for enhancing alertness and performance compared to bright light exposure, especially for tasks requiring cognitive effort. The ability of caffeine to enhance both tasks with and without memory components is an important finding since performance during sustained or continuous operations degrades fastest in leadership positions (Anderson, 1988-89).

There are distinct advantages and disadvantages of exposure to bright light and of caffeine ingestion. For example, the use of bright light allows control over alertness and performance (Badia et al., 1991). When enhanced alertness/performance is required, photic stimulation can be introduced whereas when sleep or rest is desired, photic stimulation can be withdrawn. A disadvantage of bright light is that continuous exposure to a bright light environment is needed. This requirement of constant stimulation can be a problem when it is impossible to provide a bright light environment due to technical or safety issues (e.g., it would be difficult to implement bright light in combat situations). Similarly, the use of caffeine has advantages and disadvantages. For example, caffeine is easy to introduce but difficult to withdraw. Caffeine in pill form is easy to administer and requires no additional effort for at least 6 hours. Furthermore, there are no technical limitations (e.g., a power source is not required) or safety concerns (e.g., an increase in visibility to the enemy does not occur) such as those noted for bright light. The inability to withdraw caffeine is a problem if sleep or rest is desired. For situations requiring sustained operations where it is possible to implement bright light treatment continuously, the

combination of bright light and caffeine is the most effective treatment for enhancing alertness and performance.

The data presented were the results of short term use of the treatments. Additional research is necessary to determine the efficacy of the treatments for long-term use.

Recommendations for Enhancing Alertness and Performance during the Nighttime Hours.

Bright light and caffeine are effective treatments for enhancing nighttime alertness and performance for short-term periods (i.e., one or two nights) of sleep deprivation.

For military positions requiring minimal cognitive effort exposure to bright light will enhance performance during the nighttime hours.

Caffeine administration appears to be a more powerful treatment for enhancing alertness and performance compared to bright light exposure, especially for tasks requiring cognitive effort.

For situations requiring sustained operations where it is possible to implement bright light treatment continuously, the combination of bright light and caffeine is the most effective treatment for enhancing alertness and performance.

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APPENDIX A

Bright Light Affects Body Temperature Affects Body Temperature and Melatonin Levels during 48 Hours of Sleep Deprivation

Introduction

Earlier we showed that photic stimulation (> 5,000 lux) had immediate effects during the nighttime hours on alertness, body temperature, EEG, and performance during 24 hr of sleep deprivation (Badia et al., 1991). Because these effects occurred only during the nighttime when melatonin is synthesized and released and with light intensities known to suppress melatonin, we assumed that melatonin was also suppressed. Additional research conducted in our laboratory and the work of others suggested that melatonin mediates the physiological and psychological effects of photic stimulation (Myers et al., 1992). In the present investigation, we tested whether bright light (> 2,500 lux) would continue to enhance temperature and suppress melatonin during 48 hr of sleep deprivation.

Method

Six male college students (average age = 20 years) were tested. Participants arrived at 2200 hr and slept from 0000 to 0900 hr prior to beginning a 48-hr period of sleep deprivation. Throughout this period, a modified constant routine procedure was implemented. That is, physical activity, light exposure, and food/beverage intake were rigorously controlled. Physical activity was minimized by having the subjects remain seated upright throughout the testing session. During the daytime (0900-2100 hr) subjects remained under dim light (< 100 lux) while during the nighttime (2100-0900 hr) subjects remained under either dim light (< 100 lux) or bright light (> 2500 lux) administered in alternating and counterbalanced 3-hr blocks. Food and

beverages were allowed only every 3 hr in similar aliquots. The primary dependent measures of the study were tympanic temperature (Intelligent Medical Systems, Carlsbad, CA) which was recorded every 60 min and salivary melatonin (RIA; Elias USA, Osceola, WI) which was sampled every 60 min. To determine if there were any differences in physical activity under the two light intensities, the participants wore an actigraph (Ambulatory Monitoring, Ardsley, NY) on their nondominant wrist.

Results

Under the alternating bright light and dim light conditions of the experiment it is clear that nocturnal exposure to bright light significantly enhanced temperature and significantly suppressed melatonin relative to dim light. This effect occurred in a similar manner on both nights. We have reported the effects of bright light during 24 hr of sleep deprivation (Badia et al., 1991); therefore, only the results of the second 24 hr will be described. The mean nocturnal temperature level for the second night of deprivation (2100-0900 hr) was 98.8 °F under bright light and 98.6 °F under dim light ($p < .05$). The mean nocturnal melatonin level was 12.32 pg/mL under bright light and 28.35 pg/mL under dim light ($p < .05$). The accompanying figures depict the average change in temperature (Figure 19) or melatonin (Figure 20) as a function of light intensity and time within block for the entire second night (2100-0900 hr). Difference scores were calculated with the first measurement of each 3 hr block serving as the zero point. This initial measurement was then subtracted from each subsequent measurement within the block. The figures illustrate an advantage of measuring melatonin as masking effects are evident in the temperature data (Myers & Badia, 1993). An additional finding was that dim light melatonin levels increased across the deprivation period. Average dim light melatonin levels

were 21.91 pg/mL during the later portion of the first 24 hr (0300-0900 hr) and 43.31 pg/mL during the later portion of the second 24 hr ($p < .05$). The actigraphic data indicated that there were no differences in physical activity under the two light intensities.

Discussion

Temperature and melatonin levels were inversely related during the deprivation period. That is, exposure to bright light was associated with an increase in temperature and a decrease in melatonin (relative to dim light) whereas exposure to dim light was associated with a decrease in temperature and an increase in melatonin (relative to bright light). These results are consistent with our previous research (Badia et al., 1991) which utilized a shorter period of sleep deprivation and it provides additional support for the notion that melatonin plays an important role in thermoregulation (Myers et al 1992). It is interesting to speculate about the finding concerning the increase in dim light melatonin levels across the deprivation period. This result may represent the likely phase delay induced by the photic stimulation: but, this possibility seems improbable since the melatonin levels obtained on the second night were considerably higher than those obtained on the first night. A more likely possibility is that this finding represents a compensatory increase in melatonin levels resulting from suppression during the first 24 hr. While it is unlikely that such a rebound was induced by the sleep deprivation alone (Armstrong et al., 1993), it may be that a rebound was induced by the combination of sleep deprivation and photic stimulation (Salin-Pascual et al.. 1988). We are testing this hypothesis.

APPENDIX B

Light Visor Study:

Effects of Alternating Bright and Dim Light on Temperature

Introduction

Using a similar design to the alternating light study presented in Appendix A, we tested whether a portable light source (a "light visor") could attenuate the nighttime decrease in temperature. This study served as a preliminary test of whether portable light visors could be used as an alternative to stationary light sources (which restrict mobility) to enhance nighttime alertness and performance.

Method

Twelve college students (6 males, 6 females) were tested. Subjects arrived at the sleep laboratory at 1900 hr and remained their until 0300 hr. Throughout the testing session, a constant routine procedure was implemented to minimize masking effects of behavioral and environmental factors such as activity level, ambient illumination, posture, meal timing, sleep-wake state, and ambient temperature (e.g., Mills et al., 1978; Czeisler et al., 1990). During the constant routine, subjects remained seated (non-recumbent). Participants were allowed to read and do homework and were kept awake by conversing with a member of the research staff. From 1900 to 2100 hr, subjects sat quietly in order to provide a baseline for temperature measurement. Tympanic temperature was acquired every 30 min (see method section of the grant for a description). From 2100-0300 hr, a protocol of 1-hr blocks of alternating dim (< 80 lux) and bright light (> 1000 lux) began. Light Visors were donated by Bio-Bright, Inc.

Results

Figure 21 presents the temperature data in difference scores with a 2100 hr baseline. As evident in the figure. temperature decreased across the night regardless of whether subjects were under dim or bright light.

Conclusion

These results are not consistent with those of the alternating study which used light boxes (Appendix A). Exposure to bright light using the light boxes showed an effect on temperature whereas exposure to bright light using the light visors showed little effect on temperature. Since the light visors had little effect on nighttime temperature. it appears unlikely that the visors will have an effect on nighttime alertness and performance. On possible explanation for these results was that it was difficult to position the light visor so that light would fall directly on the eyes. This preliminary study suggests further development of the light visor is needed if it is to be used as a treatment to enhance nighttime alertness and performance.

APPENDIX C

Reliability of Salivary Melatonin

Introduction

Melatonin (MT) can be measured in saliva and serum but due to saliva's ease of collection it may be the preferred method. Given the latter, it is important that the reliability of salivary MT within individuals across multiple nights be assessed. Previous research indicates that the nightly MT rhythm in saliva is highly correlated with MT levels in blood and that salivary MT levels are approximately 30% of blood MT levels (McIntyre et al., 1987, Nowak et al., 1987). Plasma (Coetzee et al., 1989, Grof et al., 1985) and serum (Arato et al., 1985) MT levels have shown high reliability across repeated testing sessions but saliva MT reliability is less well established. Therefore, we investigated the reliability of the salivary MT profile across successive weeks.

Methods

Seven subjects (6 males, 1 female, age range 20-26 years) participated on 3 evenings, with each successive testing session separated by at least 1 week but not more than 2 weeks. Subjects adhered to the following restrictions prior to participation: no medications of any kind for 72 hrs; no alcohol for 24 hrs; no caffeine for 6 hrs; and no food for 2 hrs. Subjects reported to the laboratory at 1930 hr at which time they completed consent and medical history forms. They were seated in comfortable chairs and movement was restricted. Light levels were ≤ 100 lux at all times. From 2000 - 0200 hr, hourly 2 ml saliva samples were collected into polystyrene tubes. Samples were then centrifuged at 2500 rpm and frozen at -20°C until assayed. Saliva

samples were packed in dry ice and shipped overnight air mail to Elias USA (Osceola, WI) for radioimmunoassay.

Results.

MT levels were quite similar across days for most subjects. Similar to previous reports, intra-individual variation was low while inter-individual variation was substantial. Figure 22 shows individual subject MT profiles for all 3 nights. Individual subjects showed a range of correlations across the 3 nights from $r = .08$ (S 05, N2 vs N3) to $r = .98$ (S 01, N1 vs N2). The average across all subjects was: N1 vs N2: $r = .98$ ($p<.001$), N1 vs N3: $r = .97$ ($p<.001$), N2 vs N3: $r = .98$ ($p<.001$).

Discussion.

Salivary MT levels were reliable across time in the present study at levels lower than serum as previously reported. MT levels in saliva appear to be a reliable marker similar to serum MT estimation.

APPENDIX D

NSAID Effects on Body Temperature and Melatonin Synthesis

Information general to all studies

All subjects were screened via a phone interview for a history of reactions to anti-inflammatory drugs, and for conditions that could predispose them to adverse reactions including ulcers, asthma, nasal polyps, gastritis, and gout. All subjects were medication-free (including NSAIDs) for a minimum 72-hr period prior to each experimental session. In addition, no caffeine was permitted for 6 hrs before NSAID administration. Subjects were also screened for regular sleep/wake schedules, napping habits, and any activities at or around time of participation that could alter their sleep patterns. Females were scheduled to participate between the second and eighth day following menses (i.e., early follicular phase) to control for differential melatonin and body temperature levels across menstrual cycle phases (20, 36). Subjects arrived at the laboratory 2 hr prior to the first temperature measurement. After giving informed consent and completing questionnaires concerning medical history and morningness/eveningness, all were required to remain seated in a comfortable chair in a dimly lit room ($\leq$ 100 lux) during the entire experimental period. Activity was restricted to reading, and social interaction was limited to necessary exchanges with the experimenter. Figure 23 depicts the protocol for each of the studies described below. The initial 2 hr served as a body temperature unmasking period, during which subjects sat quietly and no measurements were obtained. At 2300 hr (nighttime studies) or 1500 hr (daytime study), baseline tympanic temperature was measured and a standard NSAID dose (650 mg aspirin, 400 mg ibuprofen; equal to manufacturer's recommended dosage) or placebo was administered with water and a small snack. The 2300 hr time of administration was chosen to occur well within the nighttime melatonin period (previous data from our laboratory

has shown that melatonin onset occurs at approximately 2100-2200 hr in this population); the 1500 hr time of administration was chosen to occur well outside the melatonin period and several hours from normal mealtimes. Both NSAID and placebo were administered in a double-blind manner in unmarked gelatin capsules. Thereafter, tympanic temperature was assessed every 15 min until 0100 hr (nighttime studies) or 1700 hr (daytime study).

NSAID and BT Studies

Nighttime/Between subjects study. Assignment to drug group was random. Each subject received a dose of aspirin, ibuprofen, or placebo. A total of 54 subjects were tested in this protocol (21 placebo [10M, 11F], 13 aspirin [6M, 7F], 20 ibuprofen [10M, 10F]).

Nighttime/Within subjects study. This experiment used only ibuprofen, given results from the between subjects study suggesting that ibuprofen had a larger effect than aspirin (along with data showing significant disruption of sleep after ibuprofen administration; Murphy et al., 1993). A total of 11 subjects were tested in this protocol (placebo/ibuprofen [10M, 1F]). The sessions were separated by a minimum 3-day washout period and a maximum of 7 days, and drug or placebo was administered in a counterbalanced order. Daytime/between subjects study. Another 17 subjects (9 placebo, 8 ibuprofen; all M) were tested during the daytime hours.

NSAIDs and Melatonin Study with Body Temperature Replication

The procedure for this study was similar to that for the nighttime studies described above. However, the experimental period was extended until 0300 hr, tympanic temperature was assessed every 30 min, and saliva samples (approximately 1200 µl unstimulated) were collected

every 60 min for purposes of radioimmunoassay of salivary melatonin levels. All subjects (N=10; 3M,7F) participated for two sessions, separated by a minimum 3-day washout period and a maximum of 7 days. Each subject received aspirin or ibuprofen at one session, and placebo at the other session in a counterbalanced order. Saliva samples were centrifuged at 2500 rpm immediately after collection, then frozen at -20°C until assayed.

Tympanic temperature was recorded using the Firsttemp system (clinical Technologies, Calabasas, CA). Because this system averages an error of ± .056°C, a minimum of two consecutive measurements differing by less than or equal to this error was required at each assessment. Saliva samples were assayed for melatonin concentration using a radioimmunoassay procedure (Elias USA, Inc., Osceola, WI).

Statistical Analysis

Difference scores were used to reduce the influence of inter-individual differences in absolute body temperature and melatonin levels, as well as to emphasize the change in body temperature or melatonin following NSAID administration. The necessity of using difference scores is illustrated by considering that in the nighttime melatonin and body temperature experiment there was a range in baseline body temperature of 36.3 - 37.6 °C, and a range in baseline melatonin levels of 1 - 36 pg/mL. Difference scores were calculated as the change from baseline just prior to NSAID administration. For example, the body temperature difference score at 2400 hr is the average difference between the body temperature levels at 2400 hr and the 2300 hr values.

Statistical tests included either repeated measures ANOVA or mixed design ANOVA (depending upon the experimental protocol) applied to difference score data for both body temperature and melatonin levels.

Results

NSAIDs and BT studies. Nighttime/Between subjects design. Body temperature changes from baseline at 2300 hr for each NSAID compared to placebo are shown in Figure 24. As predicted, the decrease in body temperature was attenuated for both NSAID groups relative to the placebo group. There were large inter-individual differences in body temperature changes, but the subjects in the placebo group generally showed a normal decline (> .4° C) in body temperature across the testing period. The average difference in body temperature at 0100 hr between the placebo group and NSAID groups was 0.11°C. A two-way ANOVA for repeated measures (Condition x Time of Night) revealed main effects for both Condition [$F(2,51)=3.34$, $p\leq.05$] and Time of Night [$F(8,408)=62.48$, $p<.05$]. Pairwise comparisons confirmed that aspirin and ibuprofen did not differ from each other, but both groups differed from placebo at every temperature assessment from 2400-0100 hr. There was also an interaction between Condition and Time of Night [$F(16,408)=2.64$, $p<.05$], further illustrating that body temperature for the NSAID groups was attenuated across the experimental period relative to body temperature in the placebo group. Within subjects design. Body temperature changes from baseline at 2300 hr after NSAID administration compared to after placebo administration are shown in Figure 25. The difference between NSAID and placebo body temperature was most evident when compared within the same individual. The average difference in body temperature at 0100 hr between the

placebo group and NSAID group was .19°C. A two-way ANOVA for repeated measures (Condition x Time of Night) revealed main effects for both Condition [$F(1,12)=7.88$, $p<.05$] and Time of Night [$F(8,80)=20.16$, $p<.001$; Greenhouse-Geisser $p<.001$]. Pairwise comparisons revealed that body temperature differed significantly between NSAID and placebo at every time point after drug administration. As expected, there was also an interaction between Condition and Time of Night [$F(8,80)=7.12$, after drug administration. As expected, there was also an interaction between Condition and Time of Night [$F(8,80)=7.12$, $p<.001$]. Daytime/between subjects design. Administration of an NSAID had no effect on body temperature during the daytime hours as shown in Figure 26. That is, when subjects were tested between 1500-1700 hr, body temperature was not different for the placebo versus the NSAID groups. Body temperature was relatively flat across the experimental period for all subjects, although some subjects in both groups showed a slight increase in temperature between 1500-1700 hr, as would be expected at this time of day when temperature is nearing its circadian peak.

NSAIDs and Melatonin Study with Body Temperature Replication.

Body Temperature. Figure 27 shows body temperature difference scores as a function of clock time after NSAID administration compared to after placebo administration. This study replicated previous findings concerning NSAID effects on body temperature as described above. All subjects exhibited the normal nighttime decrease in body temperature under the placebo condition, although some subjects showed a greater decrease in temperature from 2300-0300 hr.

NSAID treatment attenuated the nighttime decrease in body temperature. There were no differences between aspirin and ibuprofen on body temperature changes; both NSAIDs

maintained body temperature relative to placebo to a similar degree. The average change in body temperature after NSAID treatment was +.028, -.006, -.050, -.118, -.134, -.151, -.213, and -.207°C at 2330, 2400, 2430, 0100, 0130, 0200, 0230, and 0300 hr, respectively. In comparison, the average change in body temperature after placebo was -.028, -.056, -.129, -.202, -.286, -.353, and -.403°C at the same times. Thus, the average difference in body temperature at 0300 hr between the placebo groups and the NSAID groups was .196°C.

Subsequently, a 2-way ANOVA for repeated measures (Condition x Time of Night) was performed to determine (a) whether NSAIDs maintained body temperature at a higher level than placebo, and (b) whether body temperature levels within subjects changed across the night (i.e., exhibited a circadian rhythm). This analysis confirmed that body temperature differed significantly between the NSAID and placebo conditions [$F(1,9)=18.87$, $p<.01$]. Pairwise comparison at each time point established that body temperature was significantly higher after NSAID relative to placebo at 0030 hr and every 30 min from 0130 - 0300 hr ($p<.05$). There was a main effect for Time of Night as well [$F(8,72)=41.77$, $p<.01$], confirming that body temperature did decline across the experimental period as expected. Furthermore, the presence of a significant Condition x Time of Night interaction [$F(8,72)=2.74$, $p<.05$] revealed that body temperature did not decline to the same degree after NSAID administration as after placebo administration.

Melatonin. Changes in melatonin levels as a function of clock time for NSAIDs compared to placebo are also shown in Figure 27. All subjects exhibited the normal nighttime circadian increase in melatonin levels during the nighttime hours under the placebo condition, although in some subjects it appeared that melatonin levels peaked by 0200 hr and lower levels were

observed at 0300 hr. There were the normally observed large interindividual differences in absolute melatonin levels, but intraindividual levels of melatonin between placebo and drug nights were stable at the pretreatment baseline measure (i.e., 2300 hr).

NSAID treatment suppressed melatonin levels relative to placebo treatment. As with body temperature, there was no significant difference in amount of melatonin suppression between aspirin and ibuprofen; both NSAIDs reduced melatonin levels by approximately 75% at 2400h. As depicted in Figure 27, the average change in melatonin levels after NSAID administration (relative to baseline measurement of 21.5 pg/mL) was -10.7, 9.3, 20.8, and 28.6 pg/mL at 2400, 0100, 0200, and 0300 hr, respectively. In comparison, the average change in melatonin levels after placebo administration (relative to baseline measurement of 21.6 pg/mL) was 25.4, 40.0, 49.8, and 64.0 pg/mL at the same times.

Subsequently, a 2-way ANOVA for repeated measures (Condition x Time of Night) was performed to determine (a) whether melatonin levels after NSAID administration differed significantly from levels after placebo administration and (b) whether melatonin levels within a subject changed across the night (i.e., exhibited a circadian rhythm). This analysis confirmed that melatonin levels differed significantly between the NSAID and placebo conditions [$F(1,9)=18.07$, $p<.01$]. Paired-comparisons analyses established that melatonin levels were significantly lower after NSAID than after placebo at 2400, 0100, and 0200 hr ($p<.05$), but were not different at 2300 hr (baseline) or 0300 hr. As expected, there was a main effect for Time of Night [$F(4,36)=5.64$, $p<.01$], with melatonin levels increasing significantly every hour ($p<.05$). The Condition x Time interaction was not significant ($p>.05$), indicating that a circadian rhythm was exhibited under both the placebo and NSAID conditions, although the amplitude of the rhythm was lower after NSAID administration.

Summary of NSAID studies on Body Temperature and Melatonin Levels. The primary purposes of these studies were to determine whether body temperature and melatonin levels were altered by the administration of a single dose of the NSAIDs aspirin and ibuprofen in humans. The normal circadian decrease in body temperature during the nighttime hours was attenuated by the administration of aspirin or ibuprofen, but daytime body temperature was not affected by NSAID administration. It was also demonstrated that melatonin was suppressed by the administration of these NSAIDs during the nighttime hours, confirming previous reports that NSAIDs can suppress melatonin synthesis in animals (Reiter, Steinlechner, & Richardson, 1985; Ritta & Cardinali, 1980) and in humans (Surrall et al., 1987). These results are compatible with the hypothesis that some of the behavioral changes associated with NSAID administration, including changes in sleep (Murphy et al., 1994), may be due to melatonin suppression and relatively higher body temperature. The lack of effects on daytime body temperature are also compatible with this hypothesis given that melatonin levels are very low during the diurnal hours.

The circadian rhythms of melatonin and body temperature are thought to be primary markers of the output of the circadian system. Based on these data, and our previous data demonstrating effects of NSAIDs on nocturnal sleep, we hypothesized that administration of NSAIDs at appropriate times during a period of sleep deprivation would have effects on performance and alertness in a manner similar to bright light. That is, administration of NSAIDs during the nocturnal hours would facilitate the maintenance of alertness and enhance performance relative to no treatment.

APPENDIX E

Method

Research participants were male graduate and under-graduate students enrolled at Bowling Green State University. Females were not tested as the phase of the menstrual cycle alters the melatonin and temperature rhythm (Rogacz et al., 1988). Participation depended upon the regularity of their sleep-wake schedule. Students were excluded from participating if their sleeping time regularly fell outside a range from 2300 to 0900 hr, if they slept less than 7 or more than 9 hr, if they had an irregular sleep-wakefulness schedule, or if they frequently napped. In addition, students were excluded if they used tobacco products, were taking medications (prescription and nonprescription), or had any significant medical or psychological conditions. Participants were requested to refrain from the intake of any medications (for 72 hr), ethanol (for 24 hr), or caffeine (for 24 hr) prior to the experiment. Participants completing the experiment were paid $120.00 for their participation. Participants were free to withdraw from the study at their discretion and were paid on a prorated basis if they did. All participants were tested as part of a larger study entitled "Facilitative Effects of Bright Light and Nonsteroidal Anti-inflammatory Drugs during Sleep Deprivation" funded by the Army Research Institute (Contract MDA 903-93-K-0002). The project received the approval of the Human Subjects Review Board (Approval H93E41FFB).

Measures

Questionnaires

Subjects were required to fill out several forms before participating in the experiment. These forms included a sleep and caffeine log (for 1 week prior to the experiment), a food/chemical sensitivities questionnaire, a medical history questionnaire, and a subject demographic

information form. Additionally, immediately before the testing session, subjects completed several forms regarding their activities during the last 24 hr. Measures taken during the experiment included circadian rhythms (endogenous melatonin and body temperature) and behavior (alertness and performance). Procedures for recording these measures are described in detail next.

Circadian Rhythms

Endogenous melatonin. Salivary melatonin was recorded in pg/mL by radioimmunoassay ("Melatonin Direct", Elias USA, Osceola, WI, USA). Specifically, melatonin was recorded by collecting 2 ml of unstimulated saliva into polystyrene tubes. These samples were immediately centrifuged and stored at < 0 °C. They were subsequently thawed and assayed for melatonin content. Saliva was sampled every 60 min throughout Experiment 1 and every 60 min during the night (2000-0800 hr) in Experiment 2. A 60-min sampling rate was chosen since it is sufficient to detect changes in melatonin (e.g., Lewy & Sack, 1989) without being arduous for the participants. The range of this assay is 1.0 to 300.0 pg/mL and its accuracy is ± 1 pg/mL.

Body temperature. Tympanic temperature was recorded in °C by infrared measurement of the temperature of tympanic membrane ("FirstTemp", Intelligent Medical Systems, Carlsbad, CA, USA). Temperature was recorded every 60 min throughout the experiment. A 60-min sampling rate was chosen to be comparable with the melatonin data. The range of this thermometer is 0.0 to 100.0 °C and its accuracy is ± 0.1 °C.

Behavior

Alertness. Alertness was assessed by both objective and subjective measures. Objective measures included the Maintenance of Wakefulness Test (MWT: Mitler et al., 1982) and time-series analysis of the EEG. The MWT required subjects to attempt to remain awake while sitting without moving in a quiet and isolated room. During this test, the EEG, EOG, and EMG of the subjects was recorded using a polysomnograph ("Model 78", Grass Instrument, Quincy, MA, USA). The test was terminated either when the subject fell asleep (i.e., exhibited Stage II Sleep for 30 s according to Rechtschaffen & Kales, 1968 criteria) or when 15 min elapsed. The EEG data for the time-series analysis were collected for 1 min with the subjects sitting without moving in a quiet and isolated room. Subjects were instructed to keep their eyes closed to avoid eye blink artifact. These data were analyzed by spectral analysis ("Rhythm", Stellate Systems, Westmount, Quebec, Canada). The Stanford Sleepiness Scale (SSS: Hoddes et al., 1973) was administered to test for subjective sleepiness. The SSS required subjects to indicate which of seven items on an increasing scale of sleepiness best described how they currently felt. All three alertness measures were taken every 3 hr during the nighttime (i.e., Experiment 1: 2100-0900 hr; Experiment 2:2000-0800 hr). The different measures were not administered sequentially to allow alertness assessment at different times within the 3-hr period. A 3-hr sampling rate was chosen since it is sufficient to detect changes in alertness and performance (Gillooly et al., 1990) without being arduous for the participants.

Performance. A battery of computer and paper-and-pencil tasks were utilized to assess cognitive performance. These tasks are listed and described in APPENDIX F. Subjects were trained on these tasks a minimum of five hours prior to the first nighttime testing in order to assure asymptotic levels of performance. In addition, if a subject was still improving on a task

(i.e., > 10% improvement from one practice trial to the next), then additional training on that task was provided. A battery of tasks was compiled from the Walter Reed Performance Assessment Battery (Thorne, 1985), the Unified Triservices Cognitive Performance Assessment Battery (personal communication, D.R. Thorne, July 23, 1990), and in-house programs based on validated performance measures. The computer-based measures included: Procedural Memory Test, Continous Recognition, Switching Task, Dual Task, and Probed Memory Recall Test (Dinges et al., 1993). The battery was divided into two parts and each section required approximately 20-30 min to complete. Paper-and-pencil tasks included: Thurstone Word Fluency Task.

Practice effects Experiment 2

As noted, subjects practiced the tasks for at least 10 hr to ensure asymptotic performance prior to assessment under the treatment conditions. Status throughput and the other select measures for the last practice trial on Day 1 were examined for condition differences using a 2 x 2 (Drug Condition x Light Condition) ANOVA test. No significant differences between conditions were observed for a significant main effect of Drug for one measure--Switching Task ($p = .066$). Examination of the planned comparisons for the latter task showed no significant differences among the groups at the last practice.

Design

Please refer to Design section in Main Body

Procedure

The testing session began at 2030 hr Thursday and continued until 1030 hr Sunday. Subjects arrived at the laboratory by 2000 hr Thursday. After giving written consent indicating their

willingness to participate in the experiment and completing the forms described above, the requirements of the ensuing experiment were explained to the subjects, they acclimatized to the laboratory, and they retired at 0030 hr. After being given the opportunity to sleep for 10 hr in a sound- and light-attenuated room, the participants were awakened and a 48-hr period of sleep deprivation began. During this period, a constant routine was enforced with no time cues being provided to the participants. The subjects remained inactive (i.e., seated) and read materials of their choice throughout the experiment. Participants were tested in pairs with limited social interaction being permitted. Photic stimulation using bright light (≥ 2,500 lux) was achieved by having the participants sit about 1 m directly in front of two cool-white light sources (Apollo Light Systems, Orem, UT, USA) Experiment 1: Subjects in Experiment 2 received bright light from four cool-white light sources (Apollo Light Systems, Orem, UT, USA; Lighting Resources, Columbus, OH, USA) 2 placed about 1 m directly in front of subjects and two placed off to the side. In addition, three 500 watt halogen lamps were used to illuminate the rest of the room. Exposure to bright light occurred from 2100 to 0900 hr-Experiment 1 and from 2000-0800 hr-Experiment 2. Otherwise, subjects were exposed to dim light (≤ 100 lux). A starting point for the exposure to bright light of 2100 or 2000 hr was chosen since it is prior to melatonin onset and a finishing point of 0900 or 0800 hr was chosen since it is after melatonin offset. Administration of NSAIDs, caffeine and placebo was double blind and occurred at 2100 and 0300 hr-Experiment 1 and at 2100 and 0200 hr-Experiment 2 each night. These times were chosen to be comparable to the exposure to bright light. The administration of two doses of NSAIDs and caffeine 6 hr apart was chosen due to the approximate 6-hr half life of the drugs (Lieberman, 1992; Surrall et al, 1987). Unauthorized sleeping, eating, and excessive movements were not permitted.

Compliance with these instructions was monitored by presence of an experimenter and by camera. Every 3 hr. subjects were allowed a snack. The snack consisted of a variety of foods and beverages totaling approximately 500 calories. Subjects were not allowed to leave the experimental room during this break unless it was necessary to use the restroom. At 1700 hr each day, several sensors (Beckman-type, silver-silver chloride electrodes) were applied to the subjects' face and scalp to record the electrical activity of the brain, eyes, and muscles. These sensors were removed at 0930 hr each day.

APPENDIX F

Performance Tasks

Table 6

List of Tasks and Measures

Dual Task	- Throughput. Control Losses. RMS. Accuracy, Speed
Switching Task	- Math Throughput. Math Reaction Time. Math Mean Correct Reaction Time. Mannequin Throughput. Mannequin Reaction Time. Mannequin Mean Correct Reaction Time.
Procedural Memory	
Basic Block	- Throughput. Accuracy, Speed
Coded Block	- Throughput. Accuracy. Speed
Reaction Time Task (Time Uncertainty Block)	- Throughput. Accuracy, Speed. %incorrect. %lapses.
Continuous Recognition	- Throughput. %correct. Reaction Time
Two-Column Addition	- Throughput. Accuracy. Speed
Digit Recall	- Throughput. Accuracy. Speed
Probed Force Memory Recall	- Strong Associates Recalled. Weak Associates Recalled
Thurstone	- Number of Words Generated
Wilkinson Four Choice Reaction Time	- Throughput. Accuracy. Speed
Modified Psychomotor Vigilance Task	- Reaction Time

Alertness and Performance: Description of Tasks

Dual Task

This is a task of divided attention. Subjects are required to perform concurrently two tasks: Unstable Tracking and Memory Search. In the tracking task, the subjects objective is to keep a cursor centered on a target area in the middle of the monitor screen. The cursor is controlled by moving the mouse. The cursor initially appears on the central target, but tends to move horizontally away from this position. The subjects try to keep it centered over the target at all times. If it reaches the boundary line, it will reappear at the target position and begin moving away again. While subjects are controlling the cursor, they are required to respond to test letters in the memory search component of the task. Subjects are shown a "memory set" that will contain two letters, which they are allowed to look at for as long as they wish. When they have memorized this set, they press one of the response keys and the tracking task begins immediately. After a few seconds, the memory set disappears and the subjects are shown a series of single test letters and must decide whether each test letter is one of the letters in the memory set. If subjects do not respond to a test letter within a certain time, the next letter will appear.

Switching Task

This is a test of attention switching requiring spatial processing and working memory. In this task, subjects must alternate between two tasks presented simultaneously. The screen is divided such that the Mannequin Task is presented on the left half of the screen, and the Mathematical Processing Task is presented on the right half. At the bottom center of the screen is a bar, used to indicate which task subjects are to perform. The current task is indicated by the solid side of the bar. In the Mannequin task, a stick-like figure is presented holding a circle in one hand, and a square in the other. At the feet of the mannequin, either a circle or a square is shown in a box.

The subjects task is to match the object in the box with the corresponding object in the mannequin's hands, and determine which hand the mannequin is holding the object in. The objects will not always be placed in the same hand and the orientation of the mannequin will change. The mannequin will be presented face forward, upright and upside down, and face backward, upright and upside down.

In Mathematical Processing Task, subjects must solve a number of simple addition and subtraction problems to determine whether the correct answer is less or greater than 5. The problems appear one at a time on the screen. Each problem requires two operations (addition and/or subtraction).

Procedural Memory - Procedural Reaction Time

Basic Block. This task is a test of mental efficiency and reaction time. Single numbers are displayed one after the other. There are four numbers [2 ,3 4, 5] to respond to: 2 and 3 represent low numbers; 4 and 5 represent high numbers. Subjects are required to respond to the high numbers with one key and low number with another. Numbers appear every 2 s.

Coded Block. This task is the similar to the basic block with the additional difficulty that the numbers are distorted.

Reaction Time Task (Time Uncertainty Block)

This test is similar to the Procedural Memory - Procedural Reaction Time basic block with additional difficulties: Numbers appear on the left or right side of the screen and subjects must respond with the corresponding hand. In addition, numbers are presented at irregular intervals.

Continuous Recognition Task

This is a test of working or short term memory. Subjects are presented with a series of two numbers, one appearing above the other. Their task is to memorize the bottom number, and decide whether the top number is the same as the bottom number that was memorized one screen earlier.

For example, if the stimuli were:

Screen 1	Screen 2	Screen 3	Screen 4
0	4	7	3
--	--	--	--
4	7	2	1

the correct responses would be:

Screen 1 -- either "same" or "different" (neither response is incorrect because there is nothing one screen back from the first screen)

Screen 2 -- "same" because the top "4" matches the bottom "4" on Screen 1.

Screen 3 -- "same" because the "7" on top matches the bottom "7" on Screen 2.

Screen 4 -- "different" because the "3" does not match the "2" on Screen 3.

Two-Column Addition

A subject-paced mental arithmetic task. Five two-digit numbers are presented simultaneously in column format in the center of the screen. The subject determines their sum as rapidly as possible and enters it from the keyboard, beginning with the hundreds digit. The column of digits disappears with the first key entry, and no aids for the carry operation are allowed.

Digit Recall

A test of short-term memory capacity. Nine random digits are displayed simultaneously in row across the center of the screen for one second. After a three second blank retention interval eight of the original nine digits are re-displayed in a different random order and the subject enters the missing digit. A given digit may appear no more than twice on each trial, although subjects are not informed of nor usually aware of this constraint.

Probed Force Memory Recall

This is a test of delayed memory recall. Subjects are presented with four word pairs. Two of the pairs have a high degree of relatedness and two have a low degree of relatedness. After the word pairs are memorized subject complete a 10 min performance vigilance test and then are tested for recall. Subjects are presented with the first of each word pair in a different order than originally presented and asked to give the matching word.

Thurstone

This is a test of word creation. Subjects are presented with a suffix and asked to write down as many words they can think of that end with that suffix. Subjects have 3 minutes to complete the task.

Wilkinson Four-Choice Serial Reaction Time

This is a simple test of continuous reaction time. The subject is presented with a box on the computer screen made up of four quadrants. A single quadrant is illuminated randomly and the subject is to press the corresponding button on the computer keyboard as quickly as possible, thereby initiating the next trial.

Modified Psychomotor Vigilance Test

This is a test of simple reaction time with a time delay between stimulus presentation. Subjects are required to respond to a circle appearing in the middle of the computer screen as quickly as possible. Stimuli appear randomly every 3 to 17 s. The task takes 10 min to complete.

Maintenance of Wakefulness Test

This is a test of the subjects ability to maintain wakefulness. Subjects are placed in a position conducive to sleep (reclined in a lazy boy chair), told to keep their eyes open and are informed that their task is to remain awake (Mitler, 1982).

Spectral Analysis of the EEG

This is a test of subjects brain activity. Subjects are required to sit still for one minute (eyes closed) while their brain activity is monitored. Higher levels of Delta and Theta EEG activity are typically associated with sleepiness or the transition to sleep and higher levels of Alpha and Beta EEG with wakefulness (e.g., Wright, Badia and Wauquier, 1995).

Stanford Sleepiness Scale -

Seven statements are displayed regarding subjective fatigue. Subjects pick the one best describing the state they are in.

APPENDIX G

Results

Experiment 1: Bright Light and NSAID Effects

Hypothesis 1: Treatment Effects on Circadian Rhythms (Melatonin and Temperature).

Hypothesis 1 was tested by measuring the effects of bright light and NSAIDs on the circadian rhythms of melatonin and temperature.

Hypothesis 1a

Hypothesis. Exposure to bright light stimulation during the nocturnal hours of Nights 1 and 2 will suppress melatonin and will attenuate the decrease in Nighttime temperature relative to exposure to dim light stimulation. A related hypothesis was that these effects will occur in a similar manner during both Nights 1 and 2.

Hypothesis 1a was statistically evaluated in the following manner and the results obtained are presented in detail on the following pages.

Statistical Evaluation. Difference scores were calculated on an individual subject basis for each Condition and for each Night by defining the melatonin/temperature raw score obtained at 2100 hr as zero and then subtracting this raw score from each subsequent melatonin raw score. This procedure produced a set of 12 difference scores for each Condition and for each Night since melatonin/temperature was sampled hourly from 2100 until 0900 hr. Difference scores were used since there were large inter-individual differences in melatonin and temperature levels. Only the data obtained during the nighttime hours, 2100-0900 hr, were used to test this and similar hypotheses, since they concern the immediate effects of photic stimulation or NSAIDs and that interval is when one of the two groups was administered a treatment. Difference scores obtained under bright light (BLN1/BLN2) were compared to those obtained under dim light

(DLN1/DLN2) with a 2 x 2 x 12 (Night x Condition x Time of Night) analysis of variance for repeated measures test (mixed design). Additional statistical tests included a 2 x 12 (Condition x Time of Night) analysis of variance for repeated measures (mixed design) conducted for each Night separately (N1: BLN1/BLN2 versus DLN1/DLN2; N1: BLN1/DLN2 versus DLN1/DLN2; N2: BLN1/BLN2 versus DLN1/DLN2) and a one-way analysis of variance (between-subjects design) conducted at each Time of Night to determine at which clock times the data obtained under bright and dim light differed. A significant Condition x Time of Night interaction (i.e., $p < .05$ with Greenhouse-Geisser degrees of freedom correction) (or a significant main effect of Condition)obtained for both Nights was considered confirmation of Hypothesis 1a provided the differences were in the predicted direction (i.e., difference scores obtained under bright light were smaller for melatonin and larger for temperature than those obtained under dim light on both Nights). A nonsignificant effect of Night in the three-way analysis was predicted and it, along with significant bright light effects on Nights 1 and 2, was considered an indication that the bright light was effective across consecutive 24-hr periods.

Results. The next sections describe the results obtained for melatonin and those obtained for temperature.

Melatonin. Table 7 presents the analysis of variance table for the Night x Condition x Time of Night test comparing the BLN1/BLN2and DLN1/DLN2 groups. As evident in the table, there was neither a significant effect of Night nor a significant interaction with Night. Table 8 presents the analysis of variance table for the Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups on Night 1. As evident in the table, there were significant Condition and Time of Night effects as well as a significant Condition x Time of Night interaction. Examination of Figure 1 reveals that the effect of Condition was the result of lower melatonin

levels under bright light. Post-hoc tests indicated that melatonin was significantly lower under bright light from 0100 until 0900 hr. Table 9 presents the analysis of variance table for the Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups on Night 2. As evident in the table, there were significant Condition and Time of Night effects. Examination of Figure 3 reveals that the effect of Condition was the result of lower melatonin levels under bright light. Post-hoc tests indicated that melatonin was significantly lower under bright light from 0100 until 0900 hr. Table 10 presents the analysis of variance table for the Condition x Time of Night test comparing the BLN1/DLN2 and DLN1/DLN2 groups on Night 1. As evident in the table, there were significant Condition and Time of Night effects was well as a significant interaction. Examination of Figure 1 reveals that the effect of Condition was the result of lower melatonin levels under bright light. Post-hoc tests indicated that melatonin was significantly lower under bright light from 0100 until 0800 hr.

Table 7

Results of the Night x Condition x Time of Night Test Comparing the Melatonin Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Both Nights

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	25840.66077	1	25840.66077	22.87	0.0002	-
ERROR	19207.88444	17	1129.87556			
NIGH	7.50359	1	7.50359	0.03	0.8726	-
NC	5.51923	1	5.51923	0.02	0.8906	-
ERROR	4815.05122	17	283.23831			
TIME	9845.59576	11	895.05416	7.51	0.0000	0.0009
TC	3092.92194	11	281.17472	2.36	0.0095	0.0979
ERROR	22291.71868	187	119.20705			
NT	1002.03265	11	91.09388	1.79	0.0578	0.1334
NTC	766.30031	11	69.66366	1.37	0.1897	0.2495
ERROR	9504.41366	187	50.82574			

Table 8

Results of the Condition x Time of Night Test Comparing the Melatonin Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Night 1.

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	12545.43852	1	12545.43852	19.97	0.0003	-
ERROR	10681.06980	17	628.29822			
TIME	4389.65786	11	399.05981	5.46	0.0000	0.0035
TC	2567.26568	11	233.38779	3.19	0.0005	0.0363
ERROR	13671.95750	187	73.11207			

Table 9

Results of the Condition x Time of Night Test Comparing the Melatonin Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Night 2.

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	13300.74148	1	13300.74148	16.95	0.0007	-
ERROR	13341.86587	17	784.81564			
TIME	6457.97056	11	587.08823	6.06	0.0000	0.0006
TC	1291.95656	11	117.45060	1.21	0.2816	0.3147
ERROR	18124.17484	187	96.92072			

Table 10

Results of the Condition x Time of Night Test Comparing the Melatonin Data in the BLN1/DLN2 and DLN1/DLN2 Groups on Night 1.

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	17946.39071	1	17946.39071	22.40	0.0002	-
ERROR	13620.65341	17	801.21491			
TIME	3998.94711	11	363.54065	4.72	0.0000	0.0065
TC	3020.89350	11	274.62668	3.57	0.0001	0.0224
ERROR	14389.09148	187	76.94701			

Temperature. Table 11 presents the analysis of variance table for the Night x Condition x Time of Night test comparing the BLN1/BLN2and DLN1/DLN2 groups. As evident in the table, there was neither a significant effect of Night nor a significant interaction with Night.

Table 11

Results of the Night x Condition x Time of Night test Comparing the Temperature Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Both Nights

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	3.97306	1	3.97306	7.69	0.0125	-
ERROR	9.30191	18	0.51677			
NIGH	0.00792	1	0.00792	0.06	0.8084	-
NC	0.00638	1	0.00638	0.05	0.8277	-
ERROR	2.35437	18	0.13080			
TIME	12.42150	11	1.12923	47.79	0.0000	0.0000
TC	0.53689	11	0.04881	2.07	0.0244	0.0911
ERROR	4.67874	198	0.02363			
NT	0.09052	11	0.00823	0.64	0.7919	0.6563
NTC	0.19690	11	0.01790	1.40	0.1774	0.2378
ERROR	2.54037	198	0.01283			

Table 12 presents the analysis of variance table for the Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups on Night 1. As evident in the table, there were

Table 12

Results of the Condition x Time of Night Test Comparing the Temperature Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Night 1

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	1.83051	1	1.83051	4.68	0.0443	-
ERROR	7.04678	18	0.39149			
TIME	6.35990	11	0.57817	34.35	0.0000	0.0000
TC	0.14273	11	0.01298	0.77	0.6687	0.5657
ERROR	3.33229	198	0.01683			

significant Condition and Time of Night effects. Examination of Figure 2 reveals that the effect of Condition was the result of higher temperature levels under bright light. Post-hoc tests indicated that temperature was significantly higher under bright light at 2300, 2400, 0100, 0300, and 0600 hr. Table 13 presents the analysis of variance table for the Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups on Night 2. As evident in the

Table 13

Results of the Condition x Time of Night Test Comparing the Temperature Data in the BLN1/BLN2 and DLN1/DLN2 Groups on Night 2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	2.14893	1	2.14893	8.39	0.0096	-
ERROR	4.60950	18	0.25608			
TIME	6.15211	11	0.55928	28.49	0.0000	0.0000
TC	0.59105	11	0.05373	2.74	0.0026	0.0271
ERROR	3.88683	198	0.01963			

table, there were significant Condition and Time of Night effects as well as a significant Condition x Time of Night interaction. Examination of Figure 4 reveals that the effect of Condition was the result of higher temperature levels under bright light. Post-hoc tests indicated that temperature was significantly higher under bright light from 0100 until 0700 hr. Table 14 presents the analysis of variance table for the Condition x Time of Night test comparing the BLN1/DLN2 and DLN1/DLN2 groups on Night 1. As evident in the table, there were significant Condition and Time of Night effects as well as a significant Condition x Time of Night interaction. Examination of Figure 2 reveals that the effect of Condition was the result of

higher temperature levels under bright light. Post-hoc tests indicated that temperature was significantly higher under bright light from 2300 until 0600 hr.

Table 14

Results of the Condition x Time of Night Test Comparing the Temperature Data in the BLN1 DLN2 and DLN1/DLN2 Groups on Night 1

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	2.66493	1	2.66493	6.83	0.0176	-
ERROR	7.01986	18	0.38999			
TIME	6.66723	11	0.57884	35.26	0.0000	0.0000
TC	0.61335	11	0.05576	3.40	0.0002	0.0096
ERROR	3.25027	198	0.01642			

Hypothesis 1b

Hypothesis. NSAID administration on Nights 1 and 2 will suppress melatonin and attenuate the nighttime decrease in temperature relative to placebo administration. A related hypothesis was that these effects will occur in a similar manner across both nights.

Hypothesis 1b was statistically evaluated in the following manner and the results obtained are presented in detail on the following pages.

Statistical Evaluation. Difference scores were calculated in a manner similar to that described for Hypothesis 1a. Difference scores obtained during NSAID administration (NSN1/NSN2) were compared to those obtained during placebo administration (DLN1/DLN2) with a 2 x 2 x 12 (Night x Condition x Time of Night) analysis of variance for repeated measures test (mixed design). Additional statistical tests included a 2 x 12 (Condition x Time of Night) analysis of variance for repeated measures (mixed design) conducted for each Night separately and a one-way analysis of variance (between-subjects design) conducted for each Time of Night to

determine at which clock times the data obtained in the two groups differed. A significant Condition x Time of interaction (i.e., $p < .05$ with Greenhouse-Geisser degrees of freedom correction) obtained for both Nights was considered confirmation of Hypothesis 1b provided the differences were in the predicted direction (i.e., difference scores obtained during NSAID administration were smaller for melatonin and larger for temperature than those obtained during placebo administration on both Nights). A nonsignificant effect of Night in the three-way analysis was predicted and it, along with significant NSAID effects on Nights 1 and 2, was considered an indication that the NSAID was effective across consecutive 24-hr periods.

Results: The next sections describe the results obtained for melatonin and those obtained for temperature.

Melatonin. Table 15 presents the analysis of variance table for the Night x Condition x Time of Night test comparing the BLN1/BLN2and DLN1/DLN2 groups. As evident in the table, there

Table 15

Results of the Night x Condition x Time of Night Test Comparing the Melatonin Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Both Nights

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	9392.24070	1	9392.24070	5.27	0.0339	-
ERROR	32068.38250	18	1781.57681			
NIGH	19.57380	1	19.57380	0.08	0.7771	-
NC	93.16574	1	93.16574	0.39	0.5386	-
ERROR	4266.72459	18	237.04025			
TIME	16025.81660	11	1456.89242	10.27	0.0000	0.0001
TC	880.41186	11	80.03744	0.56	0.8561	0.6092
ERROR	28078.98649	198	141.81306			
NT	671.67262	11	61.06115	1.18	0.2997	0.3239
NTC	1148.78544	11	104.43504	2.03	0.0277	0.0880
ERROR	10205.88654	198	51.54488			

Table 16

Results of the Condition x Time of Night Test Comparing the Melatonin Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Night 1

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	3807.27012	1	3807.27012	3.72	0.0696	-
ERROR	18405.73309	18	1022.54073			
TIME	9826.95341	11	893.35940	8.69	0.0000	0.0000
TC	1227.62473	11	111.60225	1.09	0.3745	0.3658
ERROR	20354.42103	198	102.80011			

was neither a significant effect of Night nor a significant interaction with Night. Table 16 presents the analysis of variance table for the Condition x Time of Night test comparing the NSN1/NSN2 and DLN1/DLN2 groups on Night 1. As evident in the table, there was a trend for a significant Condition effect and there was a significant Time of Night effect. Examination of Figure 5 reveals that the effect of Condition was the result of lower melatonin levels after the administration of NSAIDs. Post-hoc tests indicated that melatonin was significantly lower after NSAIDs intake at 0200 hr. Table 17 presents the analysis of variance table for the Condition x Time of Night test comparing the NSN1/NSN2 and DLN1/DLN2 groups on Night 2. As evident

Table 17

Results of the Condition x Time of Night Test Comparing the Melatonin Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Night 2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	5678.13632	1	5678.13632	5.70	0.0281	-
ERROR	17929.37400	18	996.07633			
TIME	6870.53581	11	624.59416	6.90	0.0000	0.0005
TC	801.57256	11	72.87023	0.80	0.6353	0.4986
ERROR	17930.45200	198	90.55784			

in the table, there were significant Condition and Time of Night effects. Examination of Figure 5 reveals that the effect of Condition was the result of lower melatonin levels after the administration of NSAIDs. Post-hoc tests indicated that melatonin was significantly lower after NSAIDs intake from 0300until 0900 hr.

Temperature. Table 18 presents the analysis of variance table for the Night x Condition x Time of Night test comparing the BLN1/BLN2and DLN1/DLN2 groups. As evident in the table, there was neither a significant effect of Night nor a significant interaction with Night. Table 19 presents the analysis of variance table for the Condition x Time of Night test comparing the NSN1/NSN2 and DLN1/DLN2 groups on Night 1. As evident in the table, there was only a significant Time of Night effect. Table 20 presents the analysis of variance table for the Condition x Time of Night test comparing the NSN1/NSN2 and DLN1/DLN2 groups on Night 2. As evident in the table, there was only a significant Time of Night effect.

Table 18

Results of the Night x Condition x Time of Night Test Comparing the Temperature Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Both Nights

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	0.00063	1	0.00063	0.00	0.9732	-
ERROR	9.79474	18	0.54415			
NIGH	0.07525	1	0.07525	0.59	0.4534	-
NC	0.08034	1	0.08034	0.63	0.4388	-
ERROR	2.30685	18	0.12816			
TIME	16.34963	11	1.48633	38.59	0.0000	0.0000
TC	0.34515	11	0.03138	0.81	0.6254	0.4731
ERROR	7.62619	198	0.03852			
NT	0.09587	11	0.00872	0.64	0.7913	0.6714
NTC	0.17359	11	0.01578	1.16	0.3155	0.3337
ERROR	2.68795	198	0.01358			

Table 19

Results of the Condition x Time of Night Test Comparing the Temperature Data NSN1/NSN2 and DLN1/DLN2 Groups on Night 1.

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	0.03337	1	0.03337	0.08	0.7837	-
ERROR	7.73398	18	0.42967			
TIME	8.46675	11	0.76970	30.83	0.0000	0.0000
TC	0.29810	11	0.02710	1.09	0.3745	0.3626
ERROR	4.94275	198	0.02496			

Table 20

Results of the Condition x Time of Night Test Comparing the Temperature Data of the NSN1/NSN2 and DLN1/DLN2 Groups on Night 1

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	0.04760	1	0.04760	0.20	0.6631	-
ERROR	4.36761	18	0.24264			
TIME	7.97875	11	0.72534	26.74	0.0000	0.0000
TC	0.22064	11	0.02006	0.74	0.6998	0.5597
ERROR	5.37139	198	0.02713			

Hypothesis 1c

Hypothesis. Bright light stimulation on Night 1 will reduce the circadian amplitude for melatonin and temperature during dim light stimulation on Night 2 relative to subjects receiving dim light stimulation on both nights. Two related hypotheses were also tested (a) bright light stimulation on Night 1 will reduce the circadian amplitude of melatonin and temperature during dim light stimulation on Night 2 to an extent similar to that induced in subjects receiving bright light stimulation on both nights and. (b) bright light stimulation on Night 1 will not affect the

circadian phase of melatonin and temperature during dim light stimulation on Night 2 relative to subjects receiving either dim or bright light stimulation on both nights.

Hypothesis 1c was statistically evaluated in the following manner and the results obtained are presented in detail on the following pages.

Statistical Evaluation. Amplitude and phase were assessed in two ways. First, difference scores were calculated on an individual subject basis for each Condition and for each Night by defining the melatonin/temperature raw score obtained at 2100 hr as zero and then subtracting this raw score from each subsequent melatonin raw score. This procedure produced a set of 12 difference scores for each Condition and for each Night since melatonin/temperature was sampled hourly from 2100 until 0900 hr. Difference scores were used since there were large inter-individual differences in melatonin and temperature levels. Only the data obtained during the nighttime hours, 2100-0900 hr, were used to test this hypothesis since this interval is when the bright light was administered on Night 1. Difference scores obtained under dim light on Night 1 following bright light on Night 2 (BLN1/DLN2) were compared to those obtained under dim light on Night 2 following dim light on Night1 (DLN1/DLN2) with a 2 x 12 (Condition x Time of Night) analysis of variance for repeated measures test (mixed design). An additional statistical test was a 2 x 12 (Condition x Time of Night) analysis of variance for repeated measures (mixed design) comparing difference scores obtained under dim light on Night 1 following bright light on Night 2 (BLN1/DLN2) and those obtained under bright light on Night 2 following bright light on Night 1 (BLN1/BLN2). A significant Condition x Time of Night interaction (i.e., $p < .05$ with Greenhouse-Geisser degrees of freedom correction) (or a significant main effect of Condition) in the BLN1/DLN2-DLN1/DLN2 analysis and a nonsignificant interaction (and a nonsignificant main effect of Condition) in the BLN1/DLN2-BLN1/BLN2

analysis were considered confirmation of Hypothesis 1c provided the differences were in the predicted direction (i.e., difference scores obtained under dim light following bright light were smaller than those obtained under dim light following dim light and similar to those obtained under bright light following bright light). Second, the amplitude and phase of the melatonin and temperature rhythms were calculated on a individual subject basis using the 0900-0900 hr data for each 24-hr period. These values were obtained using the method of Brown and Czeisler (1992). This method is based on the least-squares regression of a cosine function (Halberg et al., 1967, 1972). That is, a cosine waveform was best-fitted to each circadian rhythm and the difference between the maximum and the minimum of that waveform (measured in pg/mL for melatonin and in °C for temperature) was the amplitude and the clock time (measured in hr) of the fitted peak of melatonin or the fitted trough of temperature was the phase. Amplitudes and phases obtained in the BLN1/DLN2 group were compared to those obtained in the DLN1/DLN2 group and to those obtained in the BLN1/BLN2 group with a two separate one-way analyses of variance tests (between-subjects design) for Condition. A significant effect of Condition (i.e., $p < .05$) for the BLN1/DLN2-DLN1/DLN2 comparison was considered confirmation of Hypothesis 1c provided the differences were in the predicted direction (i.e., average amplitude following bright light was less than that following dim light).

Results. The next sections describe the results obtained for melatonin and those obtained for temperature.

Melatonin. Table 21 presents the results of the difference score analysis. As evident in the table, the data obtained on Night 2 in the BLN1/DLN2 group was not different from that obtained in DLN1/DLN2 group but was different from that obtained in theBLN1/BLN2 group.

Examination of Figure 7 reveals that the latter effect was the result of melatonin being lower under bright light on Night 2 than under dim light on Night 2.

Table 21

Results of the Condition x Time of Night Test Comparing the Melatonin Difference Scores Obtained in (a) the BLN1/DLN2 and DLN1 DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1 BLN2 Groups on Night 2

BLN1/DLN2 versus DLN1/DLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	1866.30928	1	1866.30928	2.00	0.1757	-
ERROR	15888.11088	17	934.59476			
TIME	10062.15122	11	914.74102	5.55	0.0000	0.0006
TC	1570.70601	11	142.79146	0.87	0.5745	0.4898
ERROR	30826.08977	187	164.84540			

BLN1/DLN2 versus BLN1/BLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	4943.00098	1	4943.00098	8.21	0.0112	-
ERROR	9638.93700	16	602.43356			
TIME	6929.78456	11	629.98041	5.31	0.0000	0.0018
TC	993.34508	11	90.30410	0.76	0.6781	0.5365
ERROR	20874.59538	176	118.60566			

Table 22 presents the results of the circadian phase analysis. As evident in the table, no significant difference between the amplitude obtained on Night 2 in the BLN1/DLN2 group (M = 12.52 pg/mL) and that obtained in the DLN1/DLN2 group (M = 20.97 pg/mL) was noted. Similarly, no significant difference between the amplitude obtained on Night 2 in the

BLN1/DLN2 group (M = 12.52 pg/mL) and that obtained in the BLN1 BLN2 group (M = 7.01 pg/mL) was noted.

Table 22

Results of the Condition Test Comparing the Circadian Amplitude of the Melatonin Rhythm Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2

BLN1/DLN2 versus DLN1/DLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.
COND	338.10675	1	338.10675	1.73	0.2053
ERROR	3314.06693	17	194.94511		

BLN1/DLN2 versus BLN1/BLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.
COND	136.89609	1	136.89609	3.95	0.0643
ERROR	554.86209	16	34.67888		

Table 23 presents the results of the circadian amplitude analysis. As evident in the table, no significant difference between the phase obtained on Night 2 in the BLN1/DLN2 group (M = 0553 hr) and that obtained in the DLN1/DLN2 group (M = 0518 hr) was noted. Similarly, no significant difference between the phase obtained on Night 2 in the BLN1/DLN2 group (M = 0553 hr) and that obtained in the BLN1/BLN2 group (M = 0704 hr) was noted.

Table 23

Results of the Condition Test Comparing the Circadian Phase of the Melatonin Rhythm Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1 DLN2 and BLN1/BLN2 Groups on Night 2

BLN1/DLN2 versus DLN1/DLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.
COND	1.64269	1	1.64269	0.50	0.4896
ERROR	55.98889	17	3.29346		

BLN1/DLN2 versus BLN1/BLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.
COND	6.19520	1	6.19520	2.30	0.1489
ERROR	43.10764	16	2.69423		

Temperature. Table 24 presents the results of the difference score analysis. As evident in the table, the data obtained on Night 2 in the BLN1/DLN2 group was not different from that obtained in BLN1/BLN2 group but was different from that obtained in theDLN1/DLN2 group. Examination of Figure 8 reveals that the latter effects were the result of temperature being higher under dim light on Night 2 following bright light on Night 1 than under dim light on Night 2 following dim light on Night 1.

Table 25 presents the results of the circadian amplitude analysis. As evident in the table, no significant difference between the amplitude obtained on Night 2 in the BLN1/DLN2 group (M = 0.54 °C) and that obtained in the DLN1/DLN2 group (M = 0.54 °C) was noted. However, a significant difference between the amplitude obtained on Night 2 in the BLN1/DLN2 group (M = 0.54 °C) and that obtained in the BLN1/BLN2 group (M = 0.27 °C) was noted.

Table 24

Results of the Condition x Time of Night Test Comparing the Temperature Difference Scores Obtained in (a) the BLN1/DLN2 and DLN1 DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2.

BLN1/DLN2 versus DLN1/DLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	12.35208	1	12.35208	5.69	0.0283	-
ERROR	39.10325	18	2.17240			
TIME	55.06267	23	2.39403	36.43	0.0000	0.0000
TC	4.60292	23	0.20013	3.05	0.0000	0.0097
ERROR	27.20275	414	0.06571			

BLN1/DLN2 versus BLN1/BLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.	G-G PROB.
COND	0.00752	1	0.00752	0.00	0.9494	-
ERROR	32.74188	18	1.81899			
TIME	45.40198	23	1.97400	31.61	0.0000	0.0000
TC	1.45298	23	0.06317	1.01	0.4490	0.4142
ERROR	25.85713	414	0.06246			

Table 25

Results of the Condition Test Comparing the Circadian Amplitude of the Temperature Rhythm Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2.

BLN1/DLN2 versus DLN1/DLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.
COND	0.00012	1	0.00012	0.00	0.9498
ERROR	0.55305	18	0.03073		

BLN1/DLN2 versus BLN1/BLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.
COND	0.34848	1	0.34848	8.70	0.0086
ERROR	0.72134	18	0.04007		

Table 26 presents the results of the circadian phase analysis. As evident in the table, no significant difference between the phase obtained on Night 2 in the BLN1/DLN2 group (M = 0630 hr) and that obtained in the DLN1/DLN2 group on Night 2 (M = 0530 hr) was noted. Similarly, no significant difference between the phase obtained on Night 2 in the BLN1/DLN2 group (M = 0630 hr) and that obtained in the BLN1/BLN2 group (M = 0627 hr) was noted.

Table 26

Results of the Condition Test Comparing the Circadian Phase of the Temperature Rhythm Obtained in (a) the BLN1/DLN2 and DLN1/DLN2 Groups on Night 2 and (b) the BLN1/DLN2 and BLN1/BLN2 Groups on Night 2

BLN1/DLN2 versus DLN1/DLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.
COND	5.00000	1	5.00000	4.19	0.0557
ERROR	21.50000	18	1.19444		

BLN1/DLN2 versus BLN1/BLN2

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	TAIL PROB.
COND	0.01250	1	0.01250	0.00	0.9463
ERROR	48.22500	18	2.67917		

Hypothesis 1d

Hypothesis. Circadian rhythms in melatonin and temperature measured under constant dim light (in the absence of masking factors) will be reliable (i.e., highly correlated) between Night 1 and Night 2.

Hypothesis 1d was statistically evaluated in the following manner and the results obtained are presented in detail on the following pages.

Statistical Evaluation. The amplitude and phase of the melatonin and temperature rhythms were determined in a manner similar to that described for Hypothesis 1c. The mesor (i.e., the mean) of the rhythms was also determined using the best-fitted cosine waveform on an individual subject basis. The average of the fitted waveform data was the mesor (measured in pg/mL for melatonin and in °C for temperature). Rhythm markers (amplitudes, phase, mesor) obtained under dim light during the first 24-hr period were compared to those obtained under dim light during the second 24-hr period with a correlation coefficient. A significant and positive correlation (i.e., $p < .05$) for each of the rhythm markers was considered confirmation of Hypothesis 1d.

Results. The next sections describe the results obtained for melatonin and those obtained for temperature.

Melatonin. Table 1 presents the results of the correlational analyses for melatonin. As evident in the table, significant positive correlations between Night 1 and Night 2 were obtained for amplitude, phase, and mesor.

Temperature. Table 2 presents the results of the correlational analyses for temperature. As evident in the table, significant positive correlations between Night 1 and Night 2 were obtained for phase and mesor but not for amplitude.

Hypothesis 2: Treatment Effects on Behavior (Alertness, Cognition, and Performance).

Hypothesis 2 was tested by measuring the effects of bright light and NSAIDs on alertness and performance. Four measures were used to assess alertness: Alpha EEG, Theta EEG, the Maintenance of Wakefulness Test (MWT), and the Stanford Sleepiness Scale (SSS). Five tasks were used to assess performance: the Continuous Recognition Task (CRT), the Dual Task (DT), the Probed Memory Recall Test (PMRT), the Procedural Memory Task (PMT), and the

Switching Task (ST). Three tasks were assessed in two ways: DT performance was assessed by control loss and throughput measures. PMT performance was assessed by basic and coded methods, and ST performance was assessed by Mannequin and Processing performance. In sum, the results of four alertness and eight performance measures will be presented.

Hypothesis 2a

Hypothesis. Exposure to bright light stimulation on Nights 1 and 2 will enhance alertness and performance relative to exposure to dim light stimulation. A related hypothesis was that these effects will occur in a similar manner across consecutive 24-hr periods.

Hypothesis 2a was statistically evaluated in the following manner and the results obtained are presented in detail on the following pages.

Statistical Evaluation. As noted, there were four data points for each Night and Condition. Alertness/performance scores obtained under bright light (BLN1/BLN2) were compared to those obtained under dim light (DLN1/DLN2) with a 2 x 2 x 12 (Night x Condition x Time of Night) analysis of variance for repeated measures test (mixed design). Additional statistical tests included a 2 x 12 (Condition x Time of Night) analysis of variance for repeated measures (mixed design) conducted for each Night separately (N1: BLN1/BLN2 versus DLN1/DLN2; N1: BLN1/DLN2 versus DLN1/DLN2; N2: BLN1/BLN2 versus DLN1/DLN2) and a one-way analysis of variance (between-subjects design) conducted for each Time of Night to determine the clock times at which the bright and dim light data differed. A significant Condition x Time of Night interaction (i.e., $p < .05$ with Greenhouse-Geisser degrees of freedom correction) obtained for both Nights was considered confirmation of Hypothesis 2a provided the differences were in the predicted direction (i.e., data obtained under bright light revealed greater alertness/performance than those obtained under dim light on both Nights). A nonsignificant

effect of Night in the three-way analysis was predicted and it, along with significant bright light effects on Nights 1 and 2, was considered an indication that the bright light was effective across consecutive 24-hr periods.

Results. The next sections describe the results obtained for alertness and those obtained for performance.

Alertness

Alpha EEG. Figure 28 depicts relative spectral power as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, alpha increased and then decreased across time on both nights and decreased from Night 1 to Night 2. Positive effects of bright light were noted as alpha in the BLN1/BLN2 group was lower than alpha in the DLN1/DLN2 group at 0130 hr on Night 2. In addition, alpha in the BLN1/DLN2 group was lower than alpha in the DLN1/DLN2 at 0130 hr on Night 1. The Night x Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups revealed a significant Night effect [$F(1,18) = 32.72$, $p < .05$]and a significant Night x Time of Night interaction [$F(3,54) = 10.69$, $p < .05$]. Other significant interactions with Night were not obtained. The Night effect resulted from alpha decreasing from Night 1 to Night 2 and the interaction resulted from different patterns of alpha during the two nights. The Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups on Night 1 revealed only a significant Time of Night effect [$F(3,54) = 12.45$, $p < .05$] with alpha increasing and then decreasing across the night. A similar analysis of the Night 2 data revealed a significant Time of Night effect [$F(3,54) = 10.98$, $p < .05$] and a significant inter-action [$F(3,54) = 3.44$, $p < .05$]. A significant Condition effect was not obtained. The Time of Night effect resulted from alpha increasing and then decreasing across the night. The inter-action

resulted from alpha being significantly lower under bright light for the second block and being significantly higher under bright light for the third block. The Condition x Time of Night test comparing the BLN1/DLN2 and DLN1/DLN2 groups on Night 1 revealed only a significant Time of Night effect [$F(3,54) = 6.08$, $p < .05$] with alpha increasing and then decreasing across the night.

Theta EEG. Figure 29 depicts relative spectral power as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, theta increased across time on both nights and as well as from Night 1 to Night 2. Positive effects of bright light were noted as theta in the BLN1/BLN2 group was lower than theta in the DLN1/DLN2 group at 0130 and 0430 hr on Night 1 as well as at 0430 hr on Night 2. In addition, theta in the BLN1/DLN2 group was lower than theta in the DLN1/DLN2 group at 0730 hr on Night 1. The Night x Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups revealed a significant Night effect [$F(1,18) = 77.21$, $p < .05$] and a significant Night x Time of Night inter-action [$F(3,54) = 7.55$, $p < .05$]. Other significant interactions with Night were not obtained. The Night effect resulted from theta increasing from Night 1 to Night 2 and the interaction resulted from different patterns of theta during the two nights. The Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups on Night 1 revealed a trend for a significant Condition effect [$F(1,18) = 3.09$, $p < .05$], a significant Time of Night effect [$F(3,54) = 13.18$, $p < .05$], and a significant interaction [$F(3,54) = 5.85$, $p < .05$]. These findings resulted from theta (a) being lower under bright light than under dim light, (b) increasing during the night, and (c) being significantly lower under bright light than under dim light during Blocks 2 and 3. A similar analysis of the Night 2 data revealed significant Condition [$F(1,18) = 4.28$, $p < .05$] and Time of Night [$F(3,54)$

= 7.01, $p < .05$] effects. A significant inter-action was not obtained. The Condition effect resulted from theta being lower under bright light than under dim light and the Time of Night effect resulted from theta increasing during the night. The Condition x Time of Night test comparing the BLN1/DLN2 and DLN1/DLN2 groups on Night 1 revealed a significant Time of Night effect [$F(3,54) = 3.37$, $p < .05$] and a significant interaction [$F(3,54) = 4.12$, $p < .05$]. A significant Condition effect was not obtained. The Time of Night effect resulted from theta increasing during the night and the interaction resulted from theta being significantly lower under bright light than under dim light only during Block 4.

Maintenance of Wakefulness Test. Figure 30 depicts Stage 2 latency as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, latencies decreased across time on both nights and as well as from Night 1 to Night 2. Positive effects of bright light were noted with longest latencies in the BLN1/BLN2 group during the latter portion of each night; however, the longest latencies were in the DLN1/DLN2 group during the early portion of Night 2. Statistical analyses of these data are not available but preliminary work indicated that there are no significant effects of interest.

Stanford Sleepiness Scale. Figure 31 depicts subjective sleepiness as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, sleepiness increased across time on both nights and as well as from Night 1 to Night 2. Positive effects of bright light were noted with lowest sleepiness in the BLN1/BLN2 and BLN1/DLN2 groups during the middle portion of Night 1. Statistical analyses of these data are not available but preliminary work indicated that there are no significant effects of interest.

Performance

Continuous Recognition Task - Throughput. Figure 32 depicts throughput as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, throughput under bright light increased across time on both nights as well as from Night 1 to Night 2 whereas throughput under dim light decreased at these times. There was little difference from Night 1 to Night 2. The Night x Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups revealed neither a significant effect of Night nor significant interactions with Night. The Condition x Time of Night tests comparing the BLN1/BLN2 and DLN1/DLN2 groups on Nights 1 and 2 revealed trends for significant interactions [Night 1: $F(3,54) = 2.86$, $p = 0.08$; Night 2: $F(3,54) = 3.22$, $p = 0.07$]. Significant main effects were not obtained. The interactions resulted from throughput increasing under bright light and decreasing under dim light within each night. Throughput was significantly higher under bright light during Blocks 3 and 4 as well as during Blocks 7 and 8. The Condition x Time of Night test comparing the BLN1/DLN2 and DLN1/DLN2 groups on Night 1 revealed no significant findings.

Dual Task - Control Losses. Figure 33 depicts number of control losses as a function of Night, Condition, and Time of Night. (The results for Block 8 are not presented because the data of several subjects were lost due to technical difficulties.) In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, control losses increased across time on both nights and as well as from Night 1 to Night 2. Positive effects of bright light were noted with control losses under bright light being consistently lower than under dim light. The Night x Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups revealed neither a significant effect of Night nor significant interactions with Night. The Condition x

Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups on Night 1 revealed only a significant Time of Night effect [$F(3,54) = 4.65$, $p < .05$] with control losses increasing across the night. A similar analysis of the Night 2 data revealed a trend for a significant Condition effect [$F(1,18) = 3.93$, $p = .06$] and a significant Time of Night effect [$F(2,36) = 6.47$, $p < .05$]. A significant interaction was not obtained. The significant findings resulted from control losses increasing across the night and from control losses under bright light being consistently lower than those under dim light. The Condition x Time of Night test comparing the BLN1/DLN2 and DLN1/DLN2 groups on Night 1 revealed significant Condition [$F(1,18) = 19.36$, $p < .05$] and Time of Night effects [$F(3,54) = 4.17$, $p < .05$] as well as a trend for a significant interaction [$F(3,54) = 3.46$, $p = .05$]. Again, these findings resulted from control losses increasing across the night and from control losses under bright light being consistently lower than those under dim light.

Dual Task - Throughput. Figure 34 depicts throughput as a function of Night, Condition, and Time of Night. (The results for Block 8 are not presented because the data of several subjects were lost due to technical difficulties.) In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, throughput decreased across time on both nights and as well as from Night 1 to Night 2. Surprisingly, throughput was lower under bright light than under dim light during the early portion of each night. However, throughput in the BLN1/DLN2 group was higher than in the DLN1/DLN2 during the later portion of Night 1. Statistical analyses of these data are not available but preliminary work indicated that there are no significant effects of interest.

Probed Memory Recall Test - Words Recalled. Figure 35 depicts number of words recalled as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2,

BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, words recalled decreased across time on both nights and as well as from Night 1 to Night 2. The Night x Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups revealed a significant effect of Night [$F(1,18) = 15.51$, $p < .05$] and a significant Night x Condition interaction [$F(1,18) = 9.07$ $p < .05$]. Other significant interactions with Night were not obtained. The Night effect resulted from throughput decreasing from Night 1 to Night 2. The interaction resulted from words recalled being lower under bright light than under dim light on Night 1 and being higher on Night 2. The Condition x Time of Night tests comparing (a) the BLN1/BLN2 and DLN1/DLN2 groups on Night 1, (b) the BLN1/BLN2 and DLN1/DLN2 groups on Night 2, and (c) the BLN1/DLN2 and DLN1/DLN2 groups on Night revealed no significant findings except a significant Time of Night effect [$F(3,54) = 4.47$, $p < .05$] in the BLN2-DLN2 comparison with throughput decreasing across the night.

Procedural Memory Task (Basic) - Throughput. Figure 36 depicts throughput as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, throughput decreased across time on both nights as well as from Night 1 to Night 2. Surprisingly, throughput was consistently lower under bright light than under dim light on Night 1. The Night x Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups revealed a significant effect of Night [$F(1,18) = 29.75$, $p < .05$] as well as significant Night x Condition [$F(1,18) = 4.59$, $p < .05$] and Night x Time of Night [$F(3,54) = 4.18$, $p < .05$] interactions. No other significant interactions with Night were obtained. The Night effect resulted from throughput decreasing from Night 1 to Night 2, the Night x Condition interaction resulted from throughput being lower under bright light than under dim light only on Night 1, the Night x Time of Night interaction resulted from a larger decrease

in throughput across Night 2. The Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups on Night 1 revealed a trend for a significant Condition effect [$F(1,18) = 4.13$, $p = .06$] and a significant interaction [$F(3,54) = 4.38$, $p < .05$]. A significant Time of Night effect was not obtained. The significant findings resulted from throughput being lower under bright light and from throughput increasing across the night under bright light but decreasing across the night under dim light. The Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups on Night 2 revealed only a significant Time of Night effect [$F(3,54) = 12.50$, $p < .05$] with throughput decreasing across the night. The Condition x Time of Night test comparing the BLN1/DLN2 and DLN1/DLN2 groups on Night 1 revealed no significant findings.

Procedural Memory Task (Coded) - Throughput. Figure 37 depicts throughput as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, throughput decreased across time on both nights as well as from Night 1 to Night 2. Surprisingly, throughput was lower under bright light than under dim light during Night 1 whereas it was higher during the later portion of Night 2. The Night x Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups revealed a significant effect of Night [$F(1,18) = 31.71$, $p < .05$] and a significant Night x Condition interaction [$F(1,18) = 5.52$, $p < .05$]. Other significant interactions with Night were not obtained. The Night effect resulted from throughput decreasing from Night 1 to Night 2. The interaction resulted from throughput being lower under bright light than under dim light on Night 1 and being higher on Night 2.The Condition x Time of Night tests comparing (a) the BLN1/BLN2 and DLN1/DLN2 groups on Night 1, (b) the BLN1/BLN2 and DLN1/DLN2 groups on Night 2, and (c) the BLN1/DLN2 and DLN1/DLN2 groups on Night 1 revealed no

significant findings except a significant Time of Night effect [$F(3,54) = 3.59$, $p < .05$] in the BLN2-DLN2 comparison with throughput decreasing across the night.

Switching Task (Mannequin) - Throughput. Figure 38 depicts throughput as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, throughput decreased across time only during Night 2 and there was little change from Night 1 to Night 2. Surprisingly, throughput was consistently lower under bright light than under dim light on both nights. The Night x Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups revealed onlya significant Night x Condition interaction [$F(1,18) = 5.65$, $p < .05$] which resulted from throughput being significantly lower under bright light than under dim light only on Night 2. The Condition x Time of Night tests comparing (a) the BLN1/BLN2 and DLN1/DLN2 groups on Night 1, (b) the BLN1/BLN2 and DLN1/DLN2 groups on Night 2, and (c) the BLN1/DLN2 and DLN1/DLN2 groups on Night 1 revealed no significant findings except a significant Time of Night effect [$F(3,54) = 8.62$, $p < .05$] in the BLN2-DLN2 comparison with throughput decreasing across the night.

Switching Task (Math Processing) - Throughput . Figure 39 depicts throughput as a function of Night, Condition, and Time of Night. In general, in the BLN1/BLN2, BLN1/DLN2 (Night 1 only), and DLN1/DLN2 groups, throughput decreased across time only during Night 2 and there was little change from Night 1 to Night 2 until the later portion of Night 2. Positive effects of bright light were noted as throughput on Night 1 in the BLN1/DLN2 group was consistently higher than in the DLN1/DLN2 group. The Night x Condition x Time of Night test comparing the BLN1/BLN2 and DLN1/DLN2 groups revealed only a significant Night x Condition interaction [$F(1,18) = 5.81$, $p < .05$] which resulted from throughput being higher

under bright light than under dim light on Night 1 but lower on Night 2. The Condition x Time of Night tests comparing (a) the BLN1/BLN2 and DLN1/DLN2 groups on Night 1, (b) the BLN1/BLN2 and DLN1/DLN2 groups on Night 2, and (c) the BLN1/BLN2 and DLN1/DLN2 groups on Night 1 revealed no significant findings except a significant Time of Night effect [$F(3,54) = 7.63$, $p < .05$] in the BLN2-DLN2 comparison with throughput decreasing across the night.

Hypothesis 2b

Hypothesis. Nonsteroidal anti-inflammatory drug administration on Nights 1 and 2 will enhance performance and alertness relative to placebo administration. A related hypothesis was that these effects will occur in a similar manner across consecutive 24-hr periods.

Hypothesis 2b was statistically evaluated in the following manner and the results obtained are presented in detail on the following pages.

Statistical Evaluation. As noted, there were four data points for each Night and Condition. Alertness/performance scores obtained during NSAID administration (NSN1/NSN2) were compared to those obtained during placebo administration (DLN1/DLN2) with a 2 x 2 x 12 (Night x Condition x Time of Night) analysis of variance for repeated measures test (mixed design). Additional statistical tests included a 2 x 12 (Condition x Time of Night) analysis of variance for repeated measures (mixed design) conducted for each Night separately and a one-way analysis of variance (between-subjects design) conducted for each Time of Night to determine the clock times at which the NSAIDs and placebo data differed. A significant Condition x Time of Night interaction (i.e., $p < .05$ with Greenhouse-Geisser degrees of freedom correction) obtained for both Nights was considered confirmation of Hypothesis 2b provided the differences were in the predicted direction (i.e., data obtained during NSAID administration

revealed greater alertness/performance than those obtained during placebo administration). A nonsignificant effect of Night in the three-way analysis was predicted and it, along with significant NSAID effects on Nights 1 and 2, was considered an indication that the bright light was effective across consecutive 24-hr periods.

Results. Figures 28 through 39 depict the alertness and performance results for the NSN1/NSN2 group. Table 27 provides the F ratios for the significant findings obtained during the statistical analysis of Hypothesis 2b. As evident in the table, either a significant effect of Night or significant interactions with Night were obtained in every comparison. In general, these findings resulted from alertness and performance decreasing from Night 1 to Night 2. Significant effects of Time of Night are also evident in Table 27. Time of Night effects were obtained for 4 of the 12 measures on Night 1 and for 12 of the 12 measures on Night 2. In general, these findings resulted from alertness and performance decreasing from the first to the last block during each night. Finally, positive effects of NSAID administration on alertness and performance are evident in the table. There was a trend for a significant Condition effect [$F(1,18) = 3.83$, $p = .07$] on the Dual Task with less control losses in the NSN1/NSN2 group than in the DLN1/DLN2 group at0430 hr on Night 2.

Hypothesis 2c

Hypothesis. Exposure to bright light stimulation on Night 1 will enhance alertness and performance during subsequent dim light stimulation on Night 2 relative to exposure to dim light stimulation on both nights: i.e. for subjects receiving bright light on Night 1 and dim light on Night 2, there will be a delayed circadian effect of bright light on alertness and performance that will carryover to Night 2. That is, subjects should perform on Night 2 under exposure to dim light in a similar manner to those receiving bright light on Night 2.

Hypothesis 2c was statistically evaluated in the following manner and the results obtained are presented in detail on the following pages.

Statistical Evaluation. Since alertness/performance were assessed every 3 hr from 2100 until 0900 hr, there were four data points. Only the data obtained during the nighttime hours, 2100-0900 hr, were used to test this and similar hypotheses, since they concern the immediate effects of photic stimulation or NSAIDs and that interval is when one of the two groups was administered a treatment. Alertness and performance data obtained on Night 2 in the BLN1/DLN2 group were compared to that obtained on Night 2 in the DLN1/DLN2 group with a 2 x 4 (Condition x Time of Night) analysis of variance for repeated measures test (between-subjects design). Additional statistical tests included a one-way analysis of variance (between-subjects design) conducted at each Time of Night to determine at which clock times the data obtained under bright and dim light differed. A significant Condition x Time of Night interaction (i.e., $p < .05$ with Greenhouse-Geisser degrees of freedom correction) was considered confirmation of Hypothesis 2c provided the differences were in the predicted direction (i.e., data obtained under dim light on Night 2 following bright light on Night 1 revealed greater alertness/performance than those obtained under dim light on Night 2 following dim light on Night 1).

Results. Figures 28 through 39 depict the alertness and performance results for the BLN1/DLN2 group. Table 28 provides the F ratios for the significant findings obtained during the statistical analysis of Hypothesis 2c. As evident in the table, significant Time of Night effects were obtained for 12 of the 12 measures on Night 2. In general, these findings resulted

Table 27

F ratios for the Significant Findings Obtained during the Statistical Analysis of Hypothesis 2b

Measure	Effect	Alpha EEG	Theta EEG	MWT	SSS	CRT	DT-CL	DT-TP	PMRT	PMT-B	PMT-C	ST-M	ST-P
Analysis													
N x C x T	N	36.52	47.33	+	+	-	-	+	38.25	28.71	27.64	-	-
	NC	-	5.05	+	+	-	3.33 (p=.08)	+	21.81	-	-	-	-
	NT	7.55	-	+	+	5.32	-	+	-	-	4.26	9.03	9.87
C x T (Night 1)	C	-	-	+	+	-	-	+	-	-	-	-	-
	T	6.25	4.58	+	+	-	6.61	+	-	5.80	-	-	-
	CT	-	-	+	+	-	-	+	-	-	-	-	-
C x T (Night 2)	C	-	-	+	+	-	3.83 (p=.07)	+	-	-	-	-	-
	T	30.09	6.30	+	+	15.70	6.87	+	4.08	13.09	9.91	20.52	12.55
	CT	-	-	+	+	-	-	+	-	-	-	-	-

Note. Nonsignificant findings are indicated by a dash (-). Analyses not performed are indicated by a cross (+). N = Night; C = Condition; T = Time.

Table 28

F ratios for the Significant Findings Obtained during the Statistical Analysis of Hypothesis 2c

Measure	Effect	Alpha EEG	Theta EEG	MWT	SSS	CRT	DT-CL	DT-TP	PMRT	PMT-B	PMT-C	ST-M	ST-P
Analysis													
C x T (Night 2)	C	-	7.78	-	-	-	18.90	-	-	-	3.15 (p=.08)	-	-
	T	13.50	8.12	17.95	14.92	14.02	18.90	12.56	4.79	14.76	9.70	11.49	7.17
	CT	-	-	3.78	-	-	3.10 (p=.07)	-	-	-	-	-	-

Note. Nonsignificant findings are indicated by a dash (-). Analyses not performed are indicated by a cross (+). N = Night; C = Condition; T = Time.

from alertness and performance decreasing from the first to the last block during Night 2. Positive effects of previous exposure to all-night photic stimulation on alertness and performance are also evident in the table. On Night 2, the BLN1/DLN2 group, relative to the DLN1/DLN2 group, had: (a) significantly lower theta EEG [$\underline{F}(1,18) = 7.78$, $\underline{p} < .05$] at 0130 and 0430 hr, (b) significantly lower control losses on the Dual Task [$\underline{F}(1,18) = 18.90$, $\underline{p} < .05$] at 2230, 0130, and 043 hr; and (c) marginally higher throughput on the Procedural Memory Task (Coded) [$\underline{F}(1,18) = 3.15$, $p = .08$] at 0130 and 0430 hr. With each of these three findings, alertness/performance in the BLN1/DLN2 group on Night 2 was not significantly different than that in the BLN1/BLN2 group on Night 2. There was one paradoxical finding: sleep latency on the MWT was significantly shorter in the BLN1/DLN2 group than it was in the DLN1/DLN2 group [$\underline{F}(3,54) = 3.78$, $\underline{p} < .05$] at 2230 hr on Night 2.

APPENDIX H

Results

Experiment 2: Bright Light and Caffeine Effects

Repeated measure ANOVAs were used to test for significant effects of Drug Condition, Light Condition, Night, and Time-of-Night on melatonin, temperature, alertness and performance measures. In addition, Pearson Product correlations between melatonin and temperature were examined.

To compensate for possible violations of repeated measure assumptions (e.g. sphericity, homogeneity of variance) and to decrease Type I error rate, Huynh Feldt Correction Factors were used in all analyses where appropriate. The standard significance level of $p \leq .05$ was used and Modified Bonferoni Correction Factors (Kepple, 1982) were used to determine significant planned comparisons.

Hypothesis 3: Treatment Effects on Circadian Rhythms (Melatonin and Temperature).

Melatonin

Melatonin data were lost for one subject in the caffeine alone condition. The following analyses begin with addressing the question of whether caffeine ingestion affects nighttime melatonin levels in humans. In addition, whether caffeine ingestion, bright light exposure or the combined treatment of bright light and caffeine have differential effects on melatonin was tested. Melatonin (area under the curve 2100-0800 hr) data were examined using a 2 x 2 x 2 (Drug Condition x Light Condition x Night) ANOVA. Analysis of the melatonin data revealed a violation of the ANOVA assumption of homogeneity of variance (e.g., variance [SEM] of melatonin levels for the placebo condition was more than three times higher than the variance in both bright light conditions on Night 2). A log transformation (Log [x+1]) of the melatonin data

was used to correct for heterogeneity of variance. Figure 40 provides area under the curve data for Log [x+1] melatonin for the treatment conditions on both Night 1 and 2. As predicted, caffeine ingestion (under dim illumination) reduced melatonin levels compared to ingestion of a placebo; i.e., melatonin levels were lower in the dim-light caffeine condition compared to the dim-light placebo condition. Exposure to bright light also reduced melatonin levels. The amount of melatonin suppression was greater in the bright-light placebo condition compared to suppression observed in the dim-light caffeine condition. As predicted, the lowest melatonin levels were observed in the combined bright-light caffeine condition. The above effects of the treatments on melatonin were observed on both nights of sleep deprivation. Analysis of variance results revealed that there were significant main effects for Drug Condition $F(1, 35) = 7.74$, $p \leq .01$ and for Light Condition $F(1, 35) = 29.72$, $p \leq .01$ No main effect for Night or interaction effects for melatonin were observed. In general, melatonin levels after ingestion of caffeine were lower compared to melatonin levels after ingestion of a placebo; melatonin levels during exposure to bright light were lower compared to melatonin levels during exposure to dim light. A Modified Bonferoni Correction for planned comparisons (DLP vs. DLC; DLP vs. BLP; DLP vs. BLC; DLC vs. BLP; DLC vs. BLC and BLP vs. BLC each night; as well as Night 1 vs. Night 2 for each treatment condition) required a $p \leq .003$ to be significant. Examination of the planned comparisons (Figure 40) revealed melatonin to be significantly lower in the dim-light caffeine condition on Night 1 (trend on Night 2); melatonin levels were significantly lower in the bright-light placebo condition and in the combined bright-light caffeine condition for both Nights 1 and 2 compared to the dim-light placebo condition (Figure 40). Furthermore, significant differences for the amount of melatonin suppression were observed between the treatments. Specifically, melatonin levels were significantly lower in the bright-light placebo condition compared to the

dim-light caffeine condition on Night 2. Additionally, the combined treatment condition produced significantly lower melatonin levels compared to both the dim-light caffeine and bright-light placebo conditions on both Nights 1 and 2 (Figure 40).

To determine whether Time-of-Night (every hour 2100-0800 hr) affected the latter results, melatonin data were analyzed using a 2 x 2 x 12 (Drug Condition x Light Condition x Time-of-Night) ANOVA for each night separately. Figure 9 provides hourly melatonin (Log[x+1]) data for the different treatment conditions on both nights. As seen, both dim-light caffeine and bright-light placebo conditions reduced melatonin levels compared to dim-light placebo, especially between 2300 and 0400 hr. Melatonin levels were lowest in the combined treatment condition of bright-light and caffeine across the entire night. Table 29 presents ANOVA summary data of all effects for melatonin (Log [x+1]) on both Night 1 and Night 2. As seen in Table 29, main effects on both nights were observed for Drug Condition, for Light Condition and for Time-of-Night. The main effect for Time-of-Night showed, as would be expected, increases in melatonin across the night. In addition to these main effects, an interaction effect for Light Condition x Time-of-Night was observed on both Night 1 and 2 of sleep deprivation. A Modified Bonferoni Correction for planned comparisons (DLP vs. DLC; DLP vs. BLP; DLP vs. BLC; DLC vs. BLP; DLC vs. BLC and BLP vs. BLC hourly each night from 2100-0800 hr) required a $p \leq .0076$ to be significant. Examination of the planned comparisons showed significantly lower melatonin levels in the dim-light caffeine or bright-light placebo conditions for several hours when compared to melatonin levels in the dim-light placebo condition. Differences between bright-light placebo and dim-light caffeine conditions were also observed. Specifically, the bright-light placebo condition showed significantly lower melatonin levels compared to the dim-light caffeine condition for several hours on Night 2. As noted, suppression of melatonin was greatest in the combined

Table 29

Analysis of Variance Summary Table for Melatonin and Temperature Data on Night 1 and Night 2

Measure	df	F	F	F	F
		Melatonin Night 1	Melatonin Night 2	Temperature Night 1	Temperature Night 2
Drug (D)	1, 35	8.48 *	5.62 *	36.91*	3.74 (.06)
Light (L)	1, 35	21.22 *	35.11 *	8.54 *	12.39 *
Time-of-Night (ToN)	11, 385	45.98 *	72.13 *	41.74 *	38.25 *
D x L	1, 35	0.04	0.30	0.73	1.12
D x ToN	11, 35	1.33	0.73	5.30 *	2.11(.065)
L x ToN	11, 35	4.48 *	11.89 *	1.21	4.45 *
D x L x ToN	11, 385	1.22	1.21	1.12	1.13

Note. Values enclosed in parentheses represent p values of trends. *p≤ 05.

bright-light caffeine condition. That is, melatonin levels in the combined bright-light caffeine condition were significantly lower than levels in the dim-light placebo condition for most of the night and significantly lower than levels in the dim-light caffeine and bright-light placebo conditions for several hours each night. In addition to the latter significant effects, a number of trends for different melatonin levels were observed (i.e., effects with p values between $p > .0076$ and $p \leq .05$). Trends are stated for each condition comparison at specific time points. Trends for a difference in melatonin levels existed between BLP vs. DLP conditions at 2300, 0400, and 0500 hr on Night 1, and at 2300 hr on Night 2; for a difference between DLC vs. DLP conditions at 0200 hr on Night 1, and at 0100 hr on Night 2; for a difference between BLC vs. DLP conditions at 0700 hr on Night 2; existed between BLP vs. DLC conditions at 0200 hr Night 1; for a difference between BLC vs. BLP at 0100 0300, 0700, and 0800 hr on Night 1, and at 0300

hr on Night 2; for a difference between BLC vs. DLC conditions at 0600, and 0800 hr on Night 1 (all $p > .0076$ and $p \leq .05$).

Temperature

The following analyses examined whether caffeine ingestion, bright light exposure or the combined treatment of bright light and caffeine have differential effects on nighttime temperature levels. The effects of the treatments on nighttime temperature are seen in Figures 10-11, & 41. The treatment effects of caffeine ingestion, bright light exposure and the combined treatment of bright light and caffeine on temperature were evaluated. Prior to analysis, temperature data were transformed into difference scores using 2000 hr as a baseline (Figure 10). The latter transformation was performed to control for differences in temperature between treatment conditions at baseline prior to treatment onset. Temperature data (average change from 2000 hr baseline) were first examined using a 2 x 2 x 2 (Drug Condition x Light Condition x Night) ANOVA. Figure 41 shows the average temperature change for the treatment conditions on both Nights 1 and 2. As predicted, the combined bright-light caffeine condition reduced the normal drop in nighttime temperature most markedly. The latter effect was observed on both nights of sleep deprivation. Temperature levels in the dim-light caffeine condition were higher than temperature levels in both the dim-light placebo and bright-light placebo conditions on Night 1. On Night 2, temperature levels in the bright-light placebo condition were higher than those in the dim-light placebo condition. Analysis of variance revealed that the main effects of Drug Condition $F(1, 36) = 18.55$, $p \leq .01$), Light Condition $F(1, 36) = 13.83$, $p \leq .01$) and an interaction of Drug Condition x Night $F(1, 36) = 9.76$, $p \leq .01$) were significant. No main effect of Night or other interaction effects for temperature were observed. In general, temperature levels after ingestion of caffeine were higher compared to temperature levels after ingestion of a

placebo; temperature levels during exposure to bright light were higher compared to temperature levels during exposure to dim light. A Modified Bonferoni Correction for planned comparisons (DLP vs. DLC; DLP vs. BLP; DLP vs. BLC; DLC vs. BLP; DLC vs. BLC and BLP vs. BLC each night; as well as Night 1 vs. Night 2 for each treatment condition) required a $p \leq .003$ to be significant. Given the latter, examination of the planned comparisons (Figure 41) show temperature levels to be significantly higher in the bright-light caffeine condition on Nights 1 and 2 compared to temperature levels in both the dim-light placebo and the bright-light placebo conditions (Figure 41). Furthermore, temperature levels in the combined bright-light caffeine condition were significantly higher compared to temperature levels in the dim light caffeine condition on Night 2; a trend for higher temperature levels in the bright-light caffeine condition was observed on Night 1.The dim-light caffeine condition showed significantly higher temperature levels compared to both the dim-light placebo and the bright-light placebo conditions on Night 1 (Figure 41). On Night 2 however, no difference in temperature levels were observed between dim-light caffeine and dim-light placebo. A trend for higher temperature levels in the bright-light placebo condition compared to temperature levels in the dim-light placebo condition was observed on Night 2.

To determine whether Time-of-Night (every hour 2100-0800 hr) had an effect, the latter results, temperature data were analyzed using a 2 x 2 x 12 (Drug Condition x Light Condition x Time-of-Night) ANOVA for each night separately. Figure 10 provides hourly temperature data (difference from 2000 hr baseline) for the different treatment conditions on both nights. As seen, temperature levels in the bright-light caffeine condition remained high across the night. The nighttime drop in temperature was greatly attenuated in the above condition. A drop in nighttime temperature was still observed for the dim-light caffeine and bright-light placebo conditions.

However, the drop in temperature was attenuated especially in the middle portion of the night. Table 29 presents ANOVA summary data of all effects for temperature on both Night 1 and Night 2. As seen in the Table, significant main effects for Light Condition and for Time-of-Night were observed on both nights. A significant main effect for Drug Condition, was observed on Night 1 (Trend on Night 2). The main effect for Time-of-Night showed, as would be expected, decreases in temperature across the night. In addition to these main effects, several significant interactions were observed: Drug Condition x Time-of-Night (Night 1, trend on Night 2), and Light Condition x Time-of-Night (Night 2). A Modified Bonferoni Correction for planned comparisons (DLP vs. DLC; DLP vs. BLP; DLP vs. BLC; DLC vs. BLP; DLC vs. BLC and BLP vs. BLC hourly each night from 2100-0800 hr) required a $p \leq .0076$ to be significant. Examination of the planned comparisons (Figure 10) showed significantly higher temperature levels in the dim-light caffeine or bright-light placebo conditions for several hours in the middle of the night when compared to temperature levels in the dim-light placebo condition. In addition, the dim-light caffeine and bright-light placebo conditions produced significantly higher temperature levels compared to each other for several hours. Higher temperature levels were observed in the dim-light caffeine condition compared to the bright-light placebo condition on Night 1, whereas on Night 2, the bright-light placebo condition showed higher temperature levels compared to dim-light caffeine. As noted, the combined treatment of bright-light and caffeine produced the largest effects on nighttime temperature. Significantly higher temperature levels in the combined bright-light caffeine condition, compared to the dim-light placebo, dim-light caffeine and bright-light placebo conditions were observed for almost all hours across the night.

In addition to the latter significant effects, a number of trends for different temperature levels were observed (i.e., effects with p values between $p > .0076$ and $p \leq .05$). Trends are stated for

each condition comparison at specific time points. Trends existed for a difference in temperature between BLP vs. DLP conditions at 2200, 0300, 0400 and 0600 hr on Night 1, and at 2300 and 0500 hr on Night 2; for a difference between DLC vs. DLP conditions at 2200 hr on Night 1, and at 2300 and 2400 hr on Night 2; for a difference between BLC vs. DLP conditions at 2200 hr on Night 2; for a difference between BLP vs. DLC conditions at 2300 and 0400 hr on Night 1, and at 0300, 0400 and 0500 hr on Night 2; for a difference between BLC vs. BLP at 2100 and 2200 hr on Night 1, and at 2400 hr on Night 2; for a difference between BLC vs. DLC conditions at 2100, and 2200 hr on Night 1, and at 2300 hr on Night 2 (all $p > .0076$ and $p \leq .05$).

Hypothesis 4: RelationshipBetween Melatonin and Temperature Levels

The following analyses address the question of whether the caffeine and bright light treatments reduced the negative inverse relationship between melatonin and temperature levels. Individual subject correlation's between melatonin and temperature (raw and transformed data) for each condition are presented in Table 4. As seen, results for the raw and transformed data are similar. Almost all subjects (transformed data) in the dim-light placebo, bright-light placebo and dim-light caffeine groups showed a significant negative correlation between melatonin and temperature. On the other hand, less than half of the individuals in the combined treatment group showed a significant correlation between melatonin and temperature levels. Averaging across individual subjects for the transformed data, yields the highest average correlation for the dim-light placebo condition (Table 4). Average correlations between melatonin and temperature were lower for the dim-light caffeine and bright-light placebo conditions relative to the dim-light placebo condition. The combined treatment of bright-light and caffeine produced the smallest average correlation between melatonin and temperature. Analysis of Variance was used to test whether the above differences between the conditions were significant. A 2 x 2 x 2 (Drug

Condition x Light Condition x Night) design was used. Prior to analysis correlation's were transformed into a Z distribution using the following formula: 1/2 log [1+r/1-r]. The transformation was necessary for the data to meet the linearity assumption of the ANOVA test. Results show significant main effects of Drug Condition $F(1, 35) = 13.89$, $p \leq .001$ and Light Condition $F(1, 35) = 4.38$, $p \leq .05$. No effect of Night or interaction effects were observed. Planned comparisons (DLP vs. DLC; DLP vs. BLP; DLP vs. BLC; DLC vs. BLP; DLC vs. BLC and BLP vs. BLC each night) with a Modified Bonferoni correction for multiple comparisons revealed significant differences between the combined caffeine bright light treatment condition and the dim-light placebo condition on both nights and between bright-light placebo and dim light placebo on Night 1 ($p \leq .004$).

Lastly, a correlation was made between the average curve of melatonin and temperature for each condition (Figure 12). As seen in Figure 12, the correlation between melatonin and temperature was high for all conditions, albeit the correlation for the caffeine conditions were lower. Results show significant negative correlation's for all conditions (all $p \leq .05$).

Hypothesis 5: Treatment Effects on Behavior (Alertness, Cognition, and Performance).

Alertness

The following analyses compare the effectiveness of the treatments for their ability to maintain nighttime alertness across two nights of sleep deprivation. Objective alertness was measured using the maintenance of wakefulness test (MWT) and spectral analysis of the EEG. Subjective alertness was measured by the Stanford Sleepiness Scale (SSS).

To begin, for each alertness measure, data were averaged into a single score for each night and analyzed using a 2 x 2 x 2 (Drug Condition x Light Condition x Night) ANOVA for each

measure. In addition, Time-of-Night effects were observed for each night separately using a 2 x 2 x 4 (Drug Condition x Light Condition x Time-of-Night) ANOVA.

Maintenance of Wakefulness Test. For the MWT, latencies to 3 continuous epochs of sleep were shorter on Night 2 compared to Night 1 for all groups (Means in minutes[SEM]: dim light placebo Night 1= 12.98[0.66], Night 2=6.61[1.05]; bright light placebo Night 1= 13.49[0.73], Night 2=6.81[1.01]; dim light caffeine Night 1= 14.40[0.60], Night 2=10.54[0.89]; combined bright light and caffeine Night 1= 15.00[0.00], Night 2=11.61[1.15]). The SEM of 0.00 for the bright-light caffeine condition on Night 1 shows that not one subject in that condition fell asleep on Night 1. When interpreting the data from the ANOVA test it is important to consider that the assumption of homogeneity of variance is broken. Caffeine alone and the combined treatment of caffeine and bright light maintained higher levels of alertness compared to dim-light placebo and bright light alone; the latter effect was especially marked on Night 2. Analysis of variance results for the MWT data show main effects of Drug Condition $F(1, 36) = 18.11$, $p \leq .01$ and Night $F(1, 36) = 113.24$, $p \leq .01$, as well as a Drug Condition x Night interaction $F(1, 36) = 9.25$, $p \leq .01$. No significant differences among conditions were observed for latency to sleep on Night 1 of sleep deprivation. There was however a trend for a shorter latency to sleep in the dim-light placebo condition compared to the bright-light caffeine condition on Night 1 ($p = .04$; A modified Bonferoni Correction Factor required a $p \leq .003$ for significance). On Night 2, latency to sleep was significantly affected by the treatment groups. Specifically, planned comparisons showed sleep latency in the dim-light placebo and bright-light placebo conditions to be significantly shorter compared to sleep latencies in the dim-light caffeine and bright-light caffeine conditions (both $p \leq .003$. No difference between the caffeine conditions on latency to

sleep was observed. Planned comparisons also revealed significantly shorter latencies to sleep for all conditions on Night 2 when compared to sleep latencies on Night 1 (all $p \leq .003$).

Time-of-Night effects were also tested. Figure 13 provides MWT data for each condition on both nights for each trial. As can be seen, caffeine alone or the combined treatment of caffeine and bright light maintained higher levels of alertness compared to dim light placebo and bright light alone for the last trial on Night 1 and for the last three trials on Night 2. Repeated measure ANOVAs for the nighttime hours showed significant main effects for Drug Condition on Night 1 $F(1, 36) = 6.50$, $p \leq .05$ and on Night 2 $F(1, 36) = 17.95$, $p \leq .001$, and for Time-of-Night on Night 1 $F(3, 108) = 11.64$, $p \leq .0001$ and on Night 2 $F(3, 108) = 20.86$, $p \leq .0001$. In addition, an interaction between Drug and Time-of-Night on Night 1 $F(3, 108) = 6.96$, $p \leq .001$ and on Night 2 $F(3, 108) = 7.25$, $p \leq .001$ of sleep deprivation were observed. In general, caffeine produced a longer latency to sleep than no caffeine. As would be expected latency to sleep shortened across the night. Planned comparisons (Figure 13) show dim-light caffeine and the combined treatment of bright-light and caffeine to exhibit significantly longer sleep latencies on both nights when compared to the dim-light placebo and bright-light placebo conditions. These latter effects were especially evident in the early morning hours.

The alerting effects of caffeine also appear to carry over into the daytime hours. That is, alertness was greater in the dim-light caffeine and bright-light caffeine conditions compared to alertness in the dim-light placebo condition for the daytime MWT. A 2 x 2 (Drug Condition x Light Condition) ANOVA was used to test for differences in alertness on the daytime MWT. The latter test occurred between 6.5 hrs and 8 hrs following the ingestion of caffeine at 0200 hrs. Analysis of variance results for the daytime MWT show a significant main effect for Drug Condition $F(1, 36) = 20.78$, $p \leq .01$. No main effect of Light Condition nor an interaction of

Drug Condition x Light Condition was observed. Planned comparisons revealed longer sleep latencies for the dim-light caffeine and the combined bright-light caffeine conditions compared to dim light placebo (both $p \leq .008$). Both caffeine groups also showed a trend for significantly longer sleep latencies compared to the bright-light placebo condition for the daytime MWT ($p = .018$ for dim-light caffeine; $p = .015$ for bright-light caffeine; a modified Bonferoni Correction of $p \leq .008$ was necessary for significance).

Spectral Analysis of the EEG. Objective alertness was also measured using power spectral analysis of EEG activity. Figures 42-43 presents data for delta, theta, alpha and beta absolute power. Little systematic effect of the treatments on EEG data were observed.

Stanford sleepiness scale (SSS). The effects of the caffeine and light treatments on subjective alertness are presented next. A higher score on the Stanford Sleepiness Scale represents more sleepiness.

Mean sleepiness [SEM] on the SSS for the treatment conditions was: dim-light placebo Night 1= 4.33[0.19], Night 2=5.65[0.24]; bright-light placebo Night 1= 4.33[0.19], Night 2=4.85[0.24]; dim-light caffeine Night 1= 3.57[0.24], Night 2=5.64[0.18]; and bright-light caffeine Night 1=2.82[0.31], Night 2=4.70[0.28]). Analysis of variance results show main effects for Drug Condition $F(1, 36) = 8.29$, $p \leq .01$, for Light Condition $F(1, 36) = 8.77$, $p \leq .01$), and for Night $F(1, 36) = 175.55$, $p \leq .01$). In addition, several significant interactions were observed: Drug Condition x Night $F(1, 36) = 23.24$, $p \leq .01$), and a Light Condition x Night $F(1, 36) = 5.18$, $p \leq .05$). Planned comparisons revealed significantly higher subjective sleepiness on Night 1 for the dim-light placebo and for the bright-light placebo conditions compared to the dim-light caffeine and bright-light caffeine conditions (both $p \leq .003$). No effect of bright-light placebo on subjective alertness was observed on Night 1. On Night 2 however, higher subjective sleepiness

was observed in the dim-light placebo condition compared to the bright-light placebo and bright-light caffeine conditions (both $p \leq .003$). The dim-light caffeine condition showed significantly higher sleepiness compared to bright-light caffeine on Night 1. On Night 2, significantly higher subjective sleepiness in the dim-light caffeine condition was observed compared to sleepiness in both the bright-light placebo and bright-light caffeine conditions. Planned comparisons also revealed significantly higher subjective sleepiness on Night 2 compared to Night 1 for the dim-light placebo, dim-light caffeine, and the combined treatment condition ($p \leq .003$). Subjects in the bright- light placebo condition showed a trend for higher subjective sleepiness on Night 2 ($p = .024$; the Modified Bonferoni correction factor required a $p < .003$ to be significant).

To determine whether Time-of-Night affected the above results, SSS data were analyzed using a 2 x 2 x 4 (Drug Condition x Light Condition x Time-of-Night) ANOVA for each night separately. Figure 14 provides SSS data for each treatment condition on both nights for each trial. As seen, the combined treatment condition shows less subjective sleepiness than dim-light placebo across the night on both nights of sleep deprivation. The dim-light caffeine condition on Night 1 and the bright-light caffeine condition on Night 2, show less subjective sleepiness across the night compared to the dim-light placebo condition. Analysis of variance shows significant main effects for Drug Condition $F(1, 36) = 22.95$, $p \leq .0001$, and for Time-of-Night $F(3, 108) = 25.26$, $p \leq .0001$ on Night 1. On Night 2, significant main effects for Light Condition $F(1, 36) = 13.46$, $p \leq .001$, for Time-of-Night $F(3, 108) = 24.57$, $p \leq .0001$) and a Light Condition x Time-of-Night interaction $F(3, 108) = 3.74$, $p \leq .05$ were observed. Planned comparisons revealed significantly less sleepiness for the combined treatment condition compared to all three individual treatments alone for a number of measurements during the night. The caffeine alone

condition on Night 1 and the bright light alone condition on Night 2 showed significantly less sleepiness compared to dim light placebo for some time points measured.

Performance Measures

The following analyses compare the effectiveness of the treatments for their ability to enhance nighttime performance across two nights of sleep deprivation.

A general summary of performance data is provided in Table 5. This table shows the percentage of time performance was better under treatment conditions compared to dim light placebo tabulated for all measures for each night of sleep deprivation. Specifically, means for each performance trial in the caffeine and bright light treatment conditions were compared to the dim light placebo condition (4 performance trials across each night of sleep deprivation = 8 total trials). If better performance occurred in the caffeine and bright light treatment conditions compared to dim light placebo a score of 1 was given to the treatment for that trial. Otherwise, if performance was the same or lower in the treatment group compared to dim light placebo a score of 0 was assigned. The total number of 1s were then tabulated for each condition separately and divided by 152 (the total number of comparisons for each night). The final data were thus a percentage of the time that performance was better under that treatment compared to dim light placebo. See Appendix F for a complete listing of all possible measures. As seen in Table 5, the combined bright-light caffeine condition, as well as the dim-light caffeine condition shows better overall performance compared to the dim light placebo condition on both nights of sleep deprivation. Bright-light placebo on the other hand, tended to enhance overall performance relative to dim-light placebo only on Night 2.

Due to the number of tasks and number of variables for each task that could be analyzed, only throughput scores and a few "select measures" were examined. "Select measures" were

analyzed because some of the tasks did not have a throughput score. Throughput is derived from accuracy and speed data yielding a measure of correct responses per minute. The throughput measure is thus sensitive to changes in accuracy and speed performance. This sensitivity of the throughput measure is useful when examining performance during sleep deprivation since when sleepy or fatigued, subjects typically slow down to increase accuracy performance or reduce accuracy to increase speed performance. Table 30 provides a list of tasks and the measure or measures analyzed for that task. Note that tasks are divided into two groupings; those with memory components and those without memory components.

Analysis of throughput measures

A general summary of the throughput and "select measures" is provided in Table 31. The table provides the percentage of the time performance was better under the bright light and caffeine conditions compared to dim-light placebo for each task averaged across all time points of the deprivation period (2130 h, 0030 h, 0330 h and 0630 h - both nights). In addition, an average was also created for time points after 0200 h (0330 h and 0630 h). The after 0200 hr comparison is of interest since performance is at it worst in the early morning hours. As seen in the table, performance in the combined treatment of bright-light and caffeine condition was 100 % of the time better than performance in the dim-light placebo group for 9 of the 13 tasks when comparing all time points tested. The number raises to 11 of 13 tasks when only the early morning hours are compared. Overall performance for the dim-light caffeine condition was 100 % of the time better relative to the dim light placebo condition for 5 of the 13 tasks. In the early

Table 30

List of Performance Tasks and Measures Analyzed

Tasks with a memory component
Dual Task - Throughput
Switching Task - Math Throughput
Reaction Time Task (Time Uncertainty Block) - Throughput
Continuous Recognition - Throughput
Two-Column Addition - Throughput
Digit Recall - Throughput
Probed Force Memory Recall - Strong Associates Recalled
Probed Force Memory Recall - Weak Associates Recalled
Thurstone - Number of Words Generated
Tasks without a memory component
Dual Task - Control Losses
Switching Task - Mannequin Throughput
Wilkinson Four Choice Reaction Time - Throughput
Modified Psychomotor Vigilance Task - Reaction Time

morning hours, the number of tasks raises to 9 of 13. Lastly, performance in the bright light alone condition was 100 % better relative to performance in the dim light placebo condition in 4 of 13 tasks for overall performance and in 5 of 13 tasks during the early morning hours. Unlike the caffeine conditions which tended to enhance performance on tasks with and without memory components, performance in the bright light alone condition tended to improve for tasks without a memory component. Specifically, performance in the bright-light placebo condition was 100 % of the time better in 4 out of 4 tasks without a memory component, whereas for tasks with a memory component bright-light placebo was better for only 1 task out of 9. On none of the tasks was performance in the dim light placebo condition 100 % of the time greater than performance

Table 31

Mean Percentage of Time for Which Performance was Better Under Treatment Compared to Placebo for Each Condition

	Bright Light Caffeine		Dim Light Caffeine		Bright Light Placebo	
TASKS WITH A MEMORY COMPONENT	All Trials	Trials After 0200 h	All Trials	Trials After 0200 h	All Trials	Trials After 0200 h
Dual Task - Throughput	**100**	**100**	**100**	**100**	**100**	**100**
Digit Recall - Throughput	**100**	**100**	**100**	**100**	**75**	**50**
Switching Task-Math - Throughput	**100**	**100**	**100**	**100**	**50**	**75**
Two-Column Addition - Throughput	**100**	**100**	**88**	**100**	**38**	**50**
Continuous Recognition - Throughput	**100**	**100**	**50**	**100**	**0**	**0**
Thurstone (Words Generated)	**88**	**75**	**63**	**75**	**63**	**75**
Reaction Time - Time Uncertainty - Throughput	**75**	**100**	**50**	**100**	**25**	**50**
Probed Force Memory Recall - Strong Associates	**50**	**75**	**50**	**75**	**50**	**50**
Probed Force Memory Recall - Weak Associates	**75**	**100**	**50**	**50**	**63**	**50**
TASKS WITHOUT A MEMORY COMPONENT						
Dual Task - Control Losses	**100**	**100**	**100**	**100**	**100**	**100**
Switching Task-Manikin - Throughput	**100**	**100**	**100**	**100**	**88**	**100**
Wilkinson - Throughtput	**100**	**100**	**88**	**100**	**100**	**100**
Psychomotor Vigilance Task - Reaction Time	**100**	**100**	**88**	**75**	**100**	**100**

in the caffeine alone or combined bright light caffeine conditions. There was however, one task in which performance of the dim-light placebo group was 100% of the time greater than performance in the bright light alone group - Continuous Recognition - Throughput.

Next, repeated measure ANOVAs were used to test for difference in performance between conditions, Night and Time-of Night. To begin, each performance task was averaged into a single score for each night and analyzed using a 2 x 2 x 2 (Drug Condition x Light Condition x Night) ANOVA. Time-of-Night effects were then tested using a 2 x 2 x 4 (Drug Condition x Light Condition x Time-of-Night) ANOVA for each night separately. Results of the ANOVA analyses for all tasks are provided in Tables 32a, 34a and 34b for tasks with a memory component. Results for tasks without a memory component are presented in Tables 32b and 34c. Four tasks showing prominent effects of the treatments are discussed in detail. Two tasks from each list of those with and without memory components were examined. The tasks with a memory component will be discussed first.

Tasks with a Memory Component. The tasks selected from the list of measures with a strong memory component are the Dual Task Throughput performance and the Switching Task Math Throughput performance.

Dual Task - Throughput. As seen in Table 32a, significant main effects for Drug Condition, for Light Condition and for Night were observed for the Dual Task Throughput performance. No significant interaction effects were observed. There was however a trend for an interaction for Drug condition x Night. In general, performance was better under caffeine versus no caffeine and better under bright light versus dim light. Performance also tended to be worse on Night 2 compared to Night 1. Table 33a presents the average performance scores for the Dual Task Throughput performance. As predicted, planned comparisons show performance to be best

in the bright-light caffeine condition. On Night 1, the bright-light caffeine condition showed significantly better performance than the dim-light placebo and dim-light caffeine conditions. On Night 2, the combined treatment condition showed significantly better performance compared to all conditions. Performance in the bright-light placebo and dim-light caffeine conditions was enhanced relative to dim-light placebo. Significantly better performance for the dim-light caffeine on Night 2 and significantly better performance for the bright-light placebo condition on both Nights 1 & 2 were observed relative to the dim-light placebo condition. No difference in performance was observed between the dim-light caffeine and bright-light placebo conditions on either night. In addition, performance for all conditions did not significantly worsen from Night 1 to Night 2. Time-of-Night effects on the above results was examined next.

Table 34a presents results of the ANOVA analysis for the Time-of-Night effects for Dual Task Throughput performance. As shown, there are significant main effects for Drug Condition on Night 2, for Light Condition on Nights 1 & 2 and for Time-of-Night on Nights 1 and 2. In addition a significant Drug x Time-of-Night effect was observed on Night 2 (Trend on Night 1). In general, performance was worse as the night progressed. That is performance, at the end of the night was worse than performance at the beginning of the night. Examination of the planned comparisons (Figure 15) show significant effects of the treatments on performance especially between 2300 and 0800 hr each night.

The bright-light placebo condition showed significantly better performance compared to the dim-light placebo condition on Night 1 for the 0030 measurement and on Night 2 for the 2130 and 0330 measurements. In addition, significantly better performance in the bright-light condition compared to the dim-light caffeine condition was observed on Night 1 for the 0030 measurement. The dim-light caffeine condition significantly improved performance over dim-

light placebo only on Night 2 for the 0630 measure. However, the bright-light caffeine condition showed the most marked effects on performance. Dual Task Throughput performance was significantly better in the bright-light caffeine condition compared to dim-light placebo for all measurements taken on both nights of sleep deprivation. Additionally, the combined treatment of bright-light and caffeine showed significantly improved performance compared to the dim-light caffeine and bright light placebo conditions for several measures each night (Figure 15).

Switching Task - Math Throughput. Table 32a shows a significant main effect for Drug Condition and a trend for Night for the Switching Task Math Throughput. No significant main effects for Light condition or for Night, nor any interaction effects were observed. In general, performance was better under caffeine versus no caffeine. The average performance scores for the Switching Task are presented in Table 33a. Planned comparisons show performance to be best in the caffeine conditions. On Nights 1 and 2, the bright-light caffeine condition and the dim-light caffeine condition showed significantly better throughput performance than the dim-light placebo and bright-light placebo conditions (Table 33a). No difference between performance in the bright-light caffeine and dim-light caffeine conditions were observed. Time-of-Night effects on the above results are examined next.

Table 34a presents results of the ANOVA analysis for the Time-of-Night effects for Switching Task performance (Math Throughput). As shown, there are significant main effects for Drug Condition on Nights 1 and 2, and Time-of-Night on Nights 1 and 2. In addition, significant Drug Condition x Time-of-Night and Light Condition x Time-of-Night interactions were observed on Night 2. In general, high levels of Math Throughout performance were maintained in the caffeine conditions across the night and performance worsened across the night in the dim-light placebo condition, especially on Night 2. Examination of the planned comparisons (Figure 15) show

significantly better performance in the caffeine conditions compared to both placebo conditions for most time points both nights. The bright-light placebo condition showed no significant effects on Math Throughput performance. No difference in performance was observed between the dim-light caffeine and bright-light caffeine conditions.

Tasks without a memory Component. Tasks selected from the list of measures without memory components include the Dual Task Control Losses performance and Wilkinson Four Choice Reaction Time Throughput performance.

Dual Task - Control Losses. As seen in Table 32b, significant main effects for Drug Condition, for Light Condition and for Night were observed for the Dual Task Control Losses performance. In addition, a significant interaction effect for Light Condition x Night was observed. No other significant interaction effects occurred. Table 33b presents the average performance for each condition on each night. The lower the control losses the better the performance. In general, performance was better under caffeine versus no caffeine and better under bright light versus dim light. Performance also tended to be worse on Night 2 compared to Night 1. As predicted, planned comparisons show the most marked effect on performance in the bright-light caffeine condition (Table 33b). On Night 1, the bright-light caffeine condition showed significantly better performance than the dim-light placebo and bright-light placebo conditions. On Night 2, the combined treatment condition showed significantly better performance compared to all conditions. Performance in the bright-light placebo and dim-light caffeine conditions was improved relative to dim-light placebo only on Night 2. No difference in performance was observed between the dim-light caffeine and bright-light placebo conditions on either night for Dual Task Control Losses performance. Time-of-Night effects on the above results was examined next.

Table 34c presents results of the ANOVA analysis for the Time-of-Night effects for Dual Task Control Loss performance. As shown, there are significant main effects for Drug Condition on Nights 1 and 2, for Light Condition on Night 2 and for Time-of-Night on Nights 1 and 2. In addition, significant Drug Condition x Time-of-Night effects were observed on Nights 1 and 2, and significant Light Condition x Time-of-Night effects was observed on Night 2. No other interaction effects were observed. In general, performance was worse as the night progressed. That is performance, at the end of the night was worse than performance at the beginning of the night. Examination of the planned comparisons (Figure 18) show significant effects of the treatments on performance especially between 2300 and 0800 hr each night. The bright-light placebo and dim-light placebo conditions significantly improved Dual Task Control Loss performance for several measurements during the early morning hours each night. That is, performance was significantly improved in both treatments compared to dim-light placebo for the 0630 measurement on Night 1 and Night 2. The bright-light placebo condition also showed significantly better performance compared to dim-light placebo for the 0330 measurement on Night 2. The bright-light caffeine condition showed significantly better performance compared to dim-light placebo for all but one measurement during sleep deprivation (Figure 18). Additionally, Dual Task Control Loss performance was significantly better in the bright-light caffeine condition compared to both the bright-light placebo and dim-light placebo conditions during the early morning hours (i.e., after 0200 hr) on both nights (Figure 18).

Wilkinson Four Choice Reaction Time - Throughput. Table 32b shows significant main effects for Light Condition and for Night on the Wilkinson Four Choice Reaction Time (Throughput) task. No significant main effect for Drug or any interaction effects were observed. In general, performance was better under bright light versus dim light and better on Night 1 than

on Night 2. The average performance scores for the Throughput measure are presented in Table 33b. Higher throughput scores represent better performance. As predicted, the bright-light caffeine condition showed the most marked effects on performance. On Nights 1 and 2, the bright-light caffeine condition showed significantly better performance compared to all other conditions. The bright-light placebo condition showed significantly better performance compared to dim-light placebo on Night 2. Performance significantly worsened on Night 2 compared to Night 1 for the dim-light placebo, dim-light caffeine and bright-light caffeine condition. Time-of-Night effects on the above results are examined next.

Table 34c presents results of the ANOVA analysis for the Time-of-Night effects for Wilkinson Four Choice Reaction Time Throughput performance. As shown, there are significant main effects for Light Condition on Night 2 (Trend on Night 1) and for Time-of-Night on both Nights 1 and 2. The effect for Drug showed a trend on Night 1. In addition to the main effects, significant interactions for Drug x Time-of-Night on Night 1 and for Light x Time of Night on Night 2 were observed. No other interaction effects occurred. In general, performance was worst during the early morning hours after 0200 hr. As predicted, performance on the Wilkinson Four Choice Reaction Time task was best in the bright-light caffeine condition (Figure 18). Examination of the planned comparisons (Figure 18) show the bright-light caffeine condition to significantly improve performance compared to the dim-light placebo condition on all but one measurement during sleep deprivation. Furthermore, performance in the bright-light caffeine condition was significantly better compared to performance in the dim-light caffeine and bright-light caffeine conditions especially after 0200 hr . The bright-light placebo condition showed significantly better performance compared to the dim-light placebo condition after 0200 hr on Nights 1 and 2. Similarly, the dim-light caffeine condition significantly improved performance

over dim-light placebo after 0200 hr but only on Night 1. No significant differences were observed on the Wilkinson task between the dim-light caffeine and bright-light placebo conditions at any time during the night.

Table 32a

Analysis of Variance Summary Table for 2 x 2 x 2 (Drug Condition x Light Condition x Night) Repeated Measures Analysis--

Tasks with a Memory Component

Task	Measure	df	F Drug (D)	F Light (L)	F Night (N)	F D x L	F D x N	F L x N	F D x L x N
Dual Task	Throughput	1,36	4.43*	9.94*	9.55*	0.00	3.00 (.09)	2.44	0.08
Switching Task-Math	Throughput	1,34	9.04*	0.29	3.97 (.054)	0.13	2.00	4.01 (.053)	2.37
Reaction Time - Time Uncertainty Block	Throughput	1,34	.65	0.58	52.21*	1.90	0.95	3.84 (.06)	1.54
Continuous Recognition	Throughput	1,36	2.30	0.63	15.84*	2.26	0.59	0.20	0.60
Two-Column Addition	Throughput	1,36	2.90	0.72	1.75	0.14	0.26	2.00	3.30
Digit Recall	Throughput	1,36	2.84	1.40	2.73	0.38	0.46	3.11 (.09)	1.07
Probed Forced Memory Recall	Strong Associates recalled	1,36	2.15	0.65	27.84*	0.74	0.05	1.48	1.07
Probed Forced Memory Recall	Weak Associates recalled	1,36	1.60	4.43*	15.35*	0.32	0.52	0.69	1.85
Thurstone	# words generated	1,36	7.55*	4.19*	1.78	4.28*	1.92	1.00	0.45

Note. Values in parentheses represent p values of trends. *$p \leq .05$

Table 32b

Analysis of Variance Summary Table for 2 x 2 x 2 (Drug Condition x Light Condition x Night) Repeated Measures Analysis--

Tasks without a Memory Component

Task	Measure	df	F Drug (D)	F Light (L)	F Night (N)	F D x L	F D x N	F L x N	F D x L x N
Dual Task	Control Losses	1,36	5.85*	4.87*	6.05*	0.05	0.55	7.04*	0.52
Switching Task- Mannequin	Throughput	1,34	7.77*	1.31	59.36*	0.23	3.43 (.07)	3.50 (.07)	0.01
Wilkinson Four Choice Reaction Time	Throughput	1,36	2.69	4.92*	39.85*	0.26	0.53	1.38	0.15
PVT - Psychomotor Vigilance Task	Reaction Time	1,34	5.68*	4.36*	73.69*	0.79	1.72	6.04*	0.35

Note. Values in parentheses represent p values of trends. *$p \leq .05$

Table 33a

Means for Performance Data: Effect of Sleep Deprivation (Night 1 vs. Night 2). Tasks with a Memory Component

	Measure	Dim Light Placebo	Bright Light Placebo	Dim Light Caffeine	Bright Light Caffeine
Dual Task	Throughput				
	Night 1	77.47	89.96 *	84.84■	96.33 *
	Night 2	67.44	84.13 * ■	79.57 * ■	97.13 *
Switching Task-Math	Throughput				
	Night 1	23.59	22.05 ■ +	27.32 *	28.86 *
	Night 2	22.00	24.26 ■ +	28.90 *	30.94 *
Reaction Time-Time Uncertainty Block	Throughput	N		N	
	Night 1	80.53	75.00 ■	76.13 ■	84.01
	Night 2	69.68	70.25 ■	69.50 ■	78.75 *
Continuous Recognition	Throughput				
	Night 1	41.74	39.13 ■	41.80 ■	50.12 *
	Night 2	44.15	41.09 ■	44.20 ■	54.67 *
Two-Column Addition	Throughput				
	Night 1	2.48	2.65	3.07 *	3.13 *
	Night 2	2.45	2.54	2.61 ■	3.26 *
Digit Recall	Throughput				
	Night 1	7.44	7.76 ■	8.41	9.32 *
	Night 2	6.78	7.41 ■	7.59 ■	9.71 *
Probed Forced Memory Recall	Strong Associates recalled	N			
	Night 1	1.73	1.60	1.73	1.85
	Night 2	1.33	1.45	1.42	1.56
Probed Forced Memory Recall	Weak Associates recalled			N	
	Night 1	1.22	1.45	1.46	1.63
	Night 2	1.03	1.17	0.96 ■	1.44
Thurstone	# words generated				
	Night 1	5.63	5.09 ■	5.29	7.10
	Night 2	4.23	4.75 ■	5.20 ■	7.23 *

Note. * = Significant difference between treatment and DLP; ■ = significant difference between BLP and BLC or DLC and BLC;

+ = significant difference between BLP and DLC; N = significant difference between Night 1 and Night 2. A $p \leq .003$ (Modified Bonferoni Correction) was used to determine significant effects.

Table 33b

Means for Performance Data: Effect of Sleep Deprivation (Night 1 vs. Night 2)— Tasks without a Memory Component

Task	Measure	Dim Light Placebo	Bright Light Placebo	Dim Light Caffeine	Bright Light Caffeine
Dual Task	Control Losses	N			
	Night 1	26.24	22.62 ■	19.51	12.62 *
	Night 2	34.05	22.50 ■ *	23.99 ■ *	12.27 *
Switching Task- Mannequin	Throughput		N	N	N
	Night 1	24.78	25.64 ■ +	29.93 ■ *	33.21 *
	Night 2	26.80	29.39 ■+	33.66 ■ *	38.89 *
Wilkinson Four Choice Reaction Time	Throughput	N		N	N
	Night 1	161.52	170.51 ■	170.43 ■	184.27 *
	Night 2	149.92	161.78 ■ *	154.80 ■	174.30 *
PVT - Psychomotor Vigilance Task	Reaction Time	N	N	N	N
	Night 1	318.96	312.49 ■	290.68	267.15 *
	Night 2	362.68	338.38 ■	352.65 ■	299.91 *

Note. * = Significant difference between treatment and DLP; ■ = significant difference between BLP and BLC or DLC and BLC;

+ = significant difference between BLP and DLC; N = significant difference between Night 1 and Night 2. A $p \leq .003$ (Modified Bonferoni Correction) was used to determine significant effects.

Table 34a

Analysis of Variance Summary Table for 2 x 2 x 4 (Drug Condition x Light Condition x Time-of-Night) Repeated Measures Analysis.

Tasks with a Memory Component

Measure	df	F	F	F	F	F
		Dual Task	Switching Task Math	Reaction Time - Time Uncertainty Block	Continuous Recognition	Two-Column Addition
		Throughput	Throughput	Throughput (n-2)	Throughput	Throughput
Drug (D)	1, 36					
Night 1		2.09	7.47 *	0.39	2.12	3.67 (.06)
Night 2		6.21 *	9.40 *	0.85	2.36	1.89
Light (L)	1, 36					
Night 1		6.35 *	0.00	0.10	0.56	0.17
Night 2		11.52 *	0.94	1.20	0.65	1.35
Time-of-Night (ToN)	3, 108					
Night 1		5.71 *	5.97 *	1.34	11.92 *	0.97
Night 2		7.23 *	9.25 *	20.99 *	4.18 *	5.92 *
D x L	1, 36					
Night 1		0.01	0.64	3.27	2.07	0.43
Night 2		0.01	0.00	0.93	2.32	0.72
D x ToN	3, 108					
Night 1		2.50 (.06)	1.67	4.44 *	3.60 *	0.18
Night 2		4.81 *	2.99 *	2.90 *	3.15 *	0.47
L x ToN	3, 108					
Night 1		0.61	2.08	1.05	0.46	1.27
Night 2		0.38	3.05 *	0.01	1.01	2.41 (.07)
D x L x ToN	3, 108					
Night 1		1.50	0.67	0.39	1.08	1.25
Night 2		1.53	2.05	2.13	0.39	0.53

Note. Values in parentheses represent p values of trends. *$p \leq .05$

Table 34b

Analysis of Variance Summary Table for 2 x 2 x 4 (Drug Condition x Light Condition x Time-of-Night) Repeated Measures Analysis.

Tasks with a Memory Component

Measure	df	F	F	F	F
		Digit Recall	Probed Force Memory Recall	Probed Force Memory Recall	Thurstone
		Throughput	Strong Associates	Weak Associates	Words Generated
Drug (D)	1, 36				
Night 1		2.22	2.14	3.21 (.08)	1.90
Night 2		3.12 (.09)	1.00	0.37	11.11*
Light (L)	1, 36				
Night 1		0.52	0.00	2.83	1.09
Night 2		2.43	1.58	3.71 (.06)	6.07*
Time-of-Night (ToN)	3, 108				
Night 1		1.48	1.19	3.94 *	1.12
Night 2		2.43 (.07)	8.53 *	8.40 *	0.99
D x L	1, 36				
Night 1		0.11	2.00	0.07	3.72 (.06)
Night 2		0.70	0.01	1.06	2.10
D x ToN	3, 108				
Night 1		2.06	1.51	0.56	0.25
Night 2		0.35	0.31	6.01 *	0.23
L x ToN	3, 108				
Night 1		0.60	0.68	0.81	2.99*
Night 2		1.42	0.66	.059	0.61
D x L x ToN	3, 108				
Night 1		0.59	0.31	1.13	0.25
Night 2		0.27	0.50	2.04	0.23

Note. Values in parentheses represent p values of trends. *$p \leq .05$

Table 34c

Analysis of Variance Summary Table for 2 x 2 x 4 (Drug Condition x Light Condition x Time-of-Night) Repeated Measures Analysis.

Tasks without a Memory Component

Measure	df	F	F	F	F
		Dual Task	Switching Task Mannequin	Wilkinson Four Choice Reaction Time	Psychomotor Vigilance Task (PVT)
		Control Losses	Throughput	Throughput	Reaction Time
Drug (D)	1, 36				
Night 1		4.54 *	6.07 *	3.67 (.06)	11.68 *
Night 2		6.13 *	8.99 *	1.64	2.28
Light (L)	1, 36				
Night 1		1.79	0.64	3.71 (.06)	1.94
Night 2		8.07 *	2.05	5.33 *	5.75 *
Time-of-Night (ToN)	3, 108				
Night 1		6.16 *	18.76 *	15.53 *	18.71 *
Night 2		9.56 *	12.46 *	18.51 *	8.62 *
D x L	1, 36				
Night 1		0.17	0	0.17	0.62
Night 2		0.00	0	0.31	0.78
D x ToN	3, 108				
Night 1		3.32 *	0	10.55 *	5.40
Night 2		3.42 *	1	1.28	0.50
L x ToN	3, 108				
Night 1		3.33 *	2 *	3.63 *	2.17
Night 2		2.15	0	2.22	1.53
D x L x ToN	3, 108				
Night 1		0.65	0.69	0.13	0.91
Night 2		0.43	0.31	1.49	0.31

Note. Values in parentheses represent p values of trends. *p≤ .05

APPENDIX I

Personnel Working on the Project Entitled "Facilitative effects on performance following modification of circadian rhythms" --Contract # MDA 903-93-K-0002

Principle Investigators
Pietro Badia, Ph.D.
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APPENDIX J

Dissertations Supported in part by Contract # MDA 903-93-K-0002

Bryan L. Myers

Dissertation Title: **"EFFECTS OF PHOTIC STIMULATION ON THE CIRCADIAN RHYTHMS OF MELATONIN AND TEMPERTURE IN HUMANS"**

Kenneth P. Wright Jr.

Preliminary Dissertation Title: **"EFFECTS OF CAFFEINE ON MELATONIN AND BODY TEMPERATURE DURING SLEEP DEPRIVATION IN HUMANS"**

Dissertation Title: **"EFFECTS OF CAFFEINE, BRIGHT LIGHT, AND THEIR COMBINATION ON MELATONIN, BODY TEMPERATURE, ALERTNESS AND PERFORMANCE DURING TWO NIGHTS OF SLEEP DEPRIVATION"**

Patricia J. Murphy

Dissertation Title: **"FACILITATIVE EFFECTS OF NIGHTTIME PHOTIC STIMULATION AND NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON PERFORMANCE AND ALERTNESS DURING EXTENDED SLEEP DEPRIVATION"**

APPENDIX K

Abstracts, Publications and Presentations supported by Contract # MDA 903-93-K-0002

Badia, P. Myers, B., & Murphy, P. (1992). Effects of nonsteroidal anti-inflammatory drugs on body temperature and melatonin. Sleep Research, 21, 365.

Murphy, P., Myers, B., Wright, K., Boecker, M., & Badia, P. (1993). Nonsteroidal anti-inflammatory drugs and melatonin levels in humans. Sleep Research, 22, 413.

Murphy, P., Badia, P., Myers, B., & Hakel, M. (1995). Nonsteroidal anti-inflammatory drug effects on melatonin and body temperature during 48 hours of sleep deprivation. Sleep Research, 24, 531.

Murphy, P., Badia, P., & Myers, B. (In Press). Nonsteroidal anti-inflammatory drugs alter body temperature and melatonin in humans. Physiology and Behavior.

Murphy, P.J., Badia, P., Wright, K.P. Jr., Boecker, M., & Hakel, M. (1995). Bright light and nonsteroidal anti-inflammatory drug effects on performance and alertness during extended sleep deprivation. Sleep Research, 24, 532.

Myers, B., Badia, P., Murphy, P., Wright, K. Jr. & Hakel, M. (1994). Bright light affects body temperature and melatonin levels during 48 hours of sleep deprivation. Sleep Research, 23, 508.

Myers, B., Badia, P., Murphy, P., Wright, K. Jr., Hughes, R., & Hakel, M. (1995). Circadian effects of photic stimulation on the melatonin and temperature rhythms in humans. Sleep Research, 24, 534.

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EFFECTS OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON BODY TEMPERATURE AND MELATONIN

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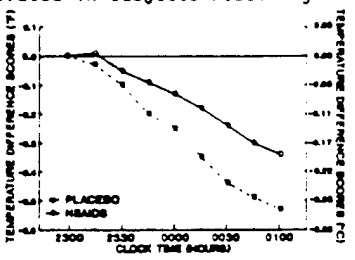
Melatonin (MEL) and body temperature (BT) have become primary markers for studying the circadian system. Our laboratory is interested in the relationship between the two measures. We recently cited evidence, both human and non-human, suggesting that MEL plays a direct role in thermoregulation[1]. Among other things, the evidence that we reviewed indicated that: (a) MEL and BT levels are inversely related; b) exogenous MEL reduces BT; and c) MEL suppression increases BT. The present report focuses on the effects of MEL suppression on BT. Suppression of MEL is usually achieved by exposure to bright light during the nighttime hours; however, other researchers have shown that MEL can also be reduced by ingestion of ibuprofen, a common nonsteroidal anti-inflammatory drug (NSAID)[2]. The latter study did not record BT. However, given that the NSAID reduced MEL levels and that MEL decreases BT, it would be expected that ingestion of ibuprofen during the nighttime hours would prevent or retard the normal drop in BT. That is, those subjects administered ibuprofen would have a higher nighttime BT than those administered placebo. In contrast, ingestion of ibuprofen during the daytime hours (when MEL levels are very low) should not affect BT. In addition to ibuprofen, the effects of two other NSAIDs (acetaminophen, aspirin) were also studied. Procedures. Male college students were screened for general health, regularity of sleep-wake schedule, and the use of medications. Since NSAIDs are known to affect endogenous prostaglandins for up to 72 hours, subjects refrained from taking NSAIDs for 72 hours prior to being tested. Throughout the experimental session, subjects were seated upright and allowed only to read under light of $\leq$ 100 lux. Subjects sat quietly for two hours prior to testing to eliminate masking effects caused by their previous activity. Tympanic temperature was assessed every 15 minutes and saliva samples were collected every hour for MEL assay. Assignment to a condition was random and double-blind. Testing sessions occurred either during the nighttime (2300 to 0100) or daytime (1500 to 1700) hours. For nighttime sessions, subjects were tested in one of four conditions: placebo (N = 15), 650 mg aspirin (N = 13), 650 mg acetaminophen (N = 11), or 400 mg ibuprofen (N = 12). For daytime sessions, subjects were tested in one of two conditions: placebo (N = 9) or 400 mg ibuprofen (N = 8). An initial BT measurement was taken immediately prior to drug administration as a baseline (zero point). Difference scores were then calculated by subtracting this initial BT measurement from each subsequent measurement. Results. During the nighttime hours, BT was elevated in subjects receiving NSAIDs relative to those receiving placebo. Analysis of variance [Condition(4) X Time(9)] revealed a significant interaction ($p < 0.05$). Specific comparisons further indicated that each of the NSAIDs maintained a significantly higher BT relative to placebo ($p < 0.05$). No significant differences between the three drugs were obtained. The accompanying figure shows BT difference scores as a function of clock time for the combined NSAID groups compared to the control group. In contrast to the nighttime hours, no effect of ibuprofen on BT during the daytime hours was noted. Conclusions. The results, combined with those of Bird and colleagues[2], indicate that MEL and BT are inversely affected by NSAIDs. This finding is compatible with our conclusion that MEL plays a direct role in thermoregulation[1]. Whether the present finding would prevail under chronic NSAID use remains to be determined. Also unknown is the temporal course of the NSAID-induced BT enhancement. Two hours after administration, BT of the subjects receiving NSAID and those receiving placebo were still significantly different. If the enhancement is controlled by prostaglandins, then BTs of the two groups should remain different as prostaglandins are significantly suppressed for up to 72 hours. However, if the effect is mediated directly by MEL, then the BT of the NSAID group should return to that of the placebo group as NSAID-induced MEL suppression appears to persist for only about 120 minutes[2]. Such findings have several implications: (a) since NSAIDs affect markers of the circadian system (MEL and BT), it may be possible to use NSAIDs to manipulate circadian rhythms; (b) since suppression of MEL and enhancement of BT by nighttime bright light increases alertness and since NSAIDs similarly affect MEL and BT, NSAIDs may also increase nighttime alertness; and (c) the effects of NSAIDs on MEL and BT may relate to their disrupting effect on sleep[3]. Since prostaglandins are known to be involved in both MEL synthesis and in thermoregulation, our results further suggest that endogenous prostaglandins may be at the beginning of the causal chain linking MEL, BT, and sleep.



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ONSTEROIDAL ANTI-INFLAMMATORY DRUG EFFECTS ON MELATONIN AND BODY TEMPERATURE DURING 48 HOURS OF SLEEP DEPRIVATION

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reviously demonstrated that single doses of the nonsteroidal anti-inflammatory drugs aspirin and ibuprofen attenuate the nighttime drop in nture (BT), and suppress melatonin (MT) synthesis in humans [1,2,3]. The mechanisms for such effects are unknown, but may involve direct consequences of inhibiting prostaglandin synthesis. Effects of NSAIDs on MT synthesis have been assessed for only 6 hr following drug n and not after multiple doses of NSAIDs. Given that MT and BT are primary indexes of circadian system output, it is important to e possible "masking" effects of these commonly used drugs. More significantly, because NSAIDs produce physiological effects similar to id during exposure to bright light, it is of considerable interest to assess whether NSAIDs alter the circadian pattern of MT and BT. col designed to determine whether NSAIDs act in a manner similar to bright light in terms of enhancing out-of-phase performance and ls (see 4.5 this volume), we assessed MT and BT levels during a 48 hr constant routine procedure. The differences between this and other which we have tested NSAID effects on MT, BT, and sleep included the timing and number of NSAID doses, and the subject's behavioral s. sleep deprivation). The primary focus of this study was to assess whether NSAIDs would affect MT and BT as previously observed stered at different phases (earlier in nighttime MT period followed by a second dose later in nighttime MT period). Also, given NSAID and MT on the first night, we wanted to determine if NSAIDs would continue to have the same physiological effects after a longer period ivation.

Twenty male subjects (mean age = 22.4 y) participated. For a minimum 72 hr prior to the experiment, none were allowed any type of dication. All were screened for history of epilepsy, irregular sleep/wake patterns (required bedtime 2300-0100 and waketime 0700-0900), Teine use, and any conditions which might preclude administration of NSAIDs (e.g., gastritis, gout, nasal polyps, sensitivity to other cipants arrived at the Sleep Laboratory at 2000 hr and slept for 10 hr prior to beginning the deprivation period. Throughout the next 48 hr onstant routine protocol was followed in which subjects' physical activity, light exposure, and food intake were rigorously controlled. ture experiment subjects remained in dim light (<100 lux); salivary MT samples were collected every 120 min and tympanic temperature every 60 min. From 2100-0900 hr each night MT sampling rate increased to every 60 min and tympanic temperature was assessed every ubjects each were randomly assigned to receive the following treatments: NS - 400 mg ibuprofen in unmarked gelatin capsules at 2100 hr ach night; PL - sugar in identical unmarked gelatin capsules at same times as NSAID administration. Saliva samples were immediately nd stored at -20 °C until assayed (elias, usa, inc., Osceola, WI).

SAID administration produced changes in BT and MT levels compared to the placebo group; however, the pattern of changes in MT and AID administration in this experiment were different from previous experiments with NSAIDs. Fig. 1 depicts BT difference scores on Night 2, calculated as the change from baseline at 2100 each night. Administration of the drug resulted in significantly higher BT levels acebo during the first half Night 1, specifically at every time point from 2230-0330 hr, except 0200 hr ($p < .05$). The trough of BT, though ase as placebo, was lower for the NS than the PL group on Night 2. Fig. 2 shows the area under the curve for melatonin (using Simpson's mula), on Night 1 and Night 2, respectively. Area under the curve for the NSAID group averaged 145 pg/ml compared to 239 pg/ml for the p on Night 1, and 119 pg/ml compared to 234 pg/ml on Night 2. A 2-way ANOVA for repeated measures (Condition x Night) confirmed ferences were significant for Condition ($F(1,18) = 4.69$, $p < .05$) and Condition x Night ($F(1,18) = 3.79$, $p < .05$). Average phase of MT rmined by the time at which salivary MT levels reached 5 pg/ml) was later for the NSAID group (2400 hr both nights) than the placebo ir Night 1; 2100 hr Night 2). Statistical analysis revealed a significant Condition x Night interaction [$F(1,18) = 3.96$, $p < .05$] for circadian tonin onset.

FIG. 1

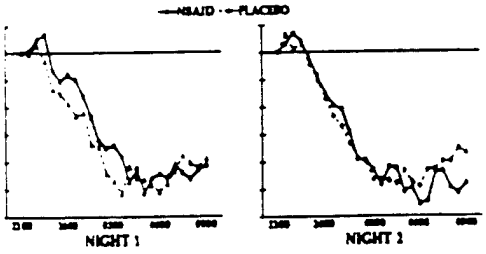
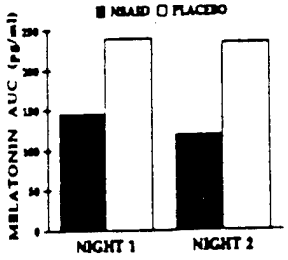


FIG. 2



s. Low doses of the NSAID ibuprofen significantly alter the circadian course of BT and suppress MT levels relative to placebo during a 48 sleep deprivation. Relatively higher BT levels were observed in the NSAID group in the first half of each night, while the BT trough was lacebo, as manifested in relatively lower BT levels during the second half of the night. MT levels were altered in an inverse manner; MT ower for the NSAID group across the entire night, and the difference in average MT levels between the PL and NS groups was magnified ompared to Night 1, possibly because further suppression of MT occurred with additional doses of the NSAID. These results are somewhat n our previous studies with NSAIDs, in that significant changes in BT and MT levels were not consistently observed within the first hour ministration. Specifically, BT levels following the 0300 NSAID dose did not attenuate the drop in BT. Reasons for this discrepancy may rences in the timing of the NSAID dose relative to MT onset or the influence of accumulating sleep deprivation. The phase of the BT s not altered by NSAID administration. MT onset phase appeared to occur later for the NSAID group on Night 1 and Night 2, but because method was used to determine phase, this finding may be misleading given that MT levels were suppressed in the NSAID group. In SAIDs can acutely alter BT and MT levels, during sleep deprivation, and multiple doses of NSAID may alter the circadian course of T and the amplitude of the MT rhythm. Further investigation of the potential phase-shifting properties of NSAIDs may be warranted.

, Sleep Res., 1992. 2. Murphy et al., Sleep Res., 1993. 3. Murphy et al., Sleep Res., 1994. 4. Murphy et al., this volume. 5. Myers et al., this volume.
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NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ALTER BODY TEMPERATURE AND SUPPRESS MELATONIN IN HUMANS

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RUNNING HEAD: NSAIDs, Body Temperature, and Melatonin

MURPHY, P. J., MYERS, B. L., and BADIA, P. *Nonsteroidal anti-inflammatory drugs alter nocturnal body temperature and suppress melatonin in humans.* PHYSIOL BEHAV 56(X): XXX-XXX, 1995. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandin synthesis in humans. Prostaglandins, in turn, are involved in thermoregulation, melatonin synthesis, and sleep. To determine effects of NSAIDs on body temperature (BT) and melatonin synthesis (MT) in humans, and to elucidate mechanisms by which NSAIDs may alter sleep patterns, a series of experiments using the NSAIDs aspirin and ibuprofen was conducted. Seventy-five subjects were tested under several experimental protocols. BT after NSAID or placebo was assessed in both between- and within-subjects designs at night and during the day. MT levels were assessed after NSAID or placebo during the night in a within-subjects design. The normal nocturnal BT decrease was attenuated and MT was suppressed after NSAID relative to after placebo administration during the nighttime hours. Lower MT levels were associated with a relative flattening of BT. Daytime BT was not affected by NSAIDs. These results are compatible with the hypothesis that some of the behavioral changes associated with NSAIDs, including changes in sleep, are due to relatively higher BT and MT suppression. We speculate that NSAID effects on sleep and BT are related to prostaglandin synthesis inhibition and/or suppression of MT.

Nonsteroidal anti-inflammatory drugs Aspirin Ibuprofen Humans
Body temperature Thermoregulation Melatonin Seasonal depression
Prostaglandins Sleep

Nonsteroidal anti-inflammatory drugs (NSAIDs) include the substances aspirin and ibuprofen. These drugs are thought to exert most of their physiological effects via prostaglandin synthesis inhibition (9,29). Prostaglandins are ubiquitous intracellular substances which directly affect sleep and body temperature (10,11,12). Prostaglandins also markedly enhance the synthesis of the pineal hormone melatonin during the nighttime hours (4,5,25,30). Given that the circadian rhythms of sleep, body temperature, and melatonin are used as primary markers of the output of the circadian system, determining potential effects of commonly used NSAIDs on these markers is important. We have previously determined that the NSAIDs aspirin and ibuprofen can change normal sleep patterns in healthy humans (14). In an attempt to further elucidate the physiological effects of commonly used NSAIDs on humans and investigate possible mechanisms of NSAID effects on sleep, we conducted a series of experiments to ascertain whether NSAIDs alter body temperature and melatonin synthesis.

Other researchers have examined NSAID effects on body temperature in humans, but results are contradictory (see 6, for a review). Most of these studies have been conducted in subjects who are febrile or in pain, in whom an immune response has most likely been initiated (9). Furthermore, NSAID type and dose level varied widely. Finally, most investigations of NSAID effects on body temperature have not evaluated time-of-day (circadian) effects. It is reasonable to predict differential circadian effects of NSAIDs on body temperature as there is a circadian rhythm in prostaglandin synthesis; prostaglandin D_2, which has hypothermic effects, is produced at higher levels in the human brain during the nighttime hours (11,17,23). Whether NSAIDs alter melatonin levels in humans has also been investigated (28). This latter study determined that a 400 mg dose of ibuprofen suppressed melatonin release by approximately 75% during the nighttime hours. However, the investigation was limited to a small number of subjects (N=4) and to males only.

Systematic studies of NSAID effects on normal body temperature and melatonin synthesis in humans with time-of-day as a factor have not been completed. Thus, the present experiments assessed how the NSAIDs aspirin and ibuprofen affect diurnal and nocturnal body temperature and nocturnal melatonin synthesis. We hypothesized that inhibiting prostaglandin synthesis by administration of aspirin and ibuprofen would attenuate the normal nocturnal decrease in body temperature and result in antagonism of nighttime melatonin synthesis. In addition, given recent reviews which illustrate the important role of melatonin in thermoregulatory processes (2,8,26) and empirical data suggesting that melatonin may be a primary regulator of body temperature (3,27), we hypothesized that body temperature and melatonin changes following NSAID administration during the nighttime hours would correspond. Specifically, we predicted that greater suppression of nighttime melatonin levels would be associated with a smaller decrease in body temperature across the experimental period.

Method

General information

All subjects were screened via a phone interview for a history of reactions to anti-inflammatory drugs, and for conditions that could predispose them to adverse reactions including ulcers, asthma, nasal polyps, gastritis, and gout. All subjects were medication-free (including NSAIDs) for a minimum 72-hr period prior to each experimental session. In addition, no caffeine was permitted for 6 hours before NSAID administration. Subjects were also screened for regular sleep/wake schedules, napping habits, and any activities at or around time of participation that could alter their sleep patterns. Females were scheduled to participate between the second and eighth day following menses (i.e., early follicular phase) to control for differential melatonin and body temperature levels across menstrual cycle phases (16,31). Subjects arrived at the laboratory 2 hr prior to the first temperature measurement. After giving informed consent and completing questionnaires concerning medical history and morningness/eveningness, all were required to remain seated in a comfortable chair in a dimly lit room (≤100 lux) during the entire

experimental period. Activity was restricted to reading, and social interaction was limited to necessary exchanges with the experimenter. The initial 2 hr served as a body temperature unmasking period. Our laboratory has shown this amount of time to be sufficient for stabilizing body temperature (15). At 2300 hr (nighttime studies) or 1500 hr (daytime study), baseline tympanic temperature was measured and a standard NSAID dose (650 mg aspirin, 400 mg ibuprofen; equal to manufacturer's recommended dosage) or placebo was administered with water and a small snack. Both NSAID and placebo were administered in a double-blind manner in unmarked gelatin capsules. Thereafter, tympanic temperature was assessed every 15 min until 0100 hr (nighttime studies) or 1700 hr (daytime study).

NSAIDs and BT studies

Nighttime/Between subjects study. Assignment to drug group was random. Each subject received a dose of aspirin, ibuprofen, or placebo. A total of 54 subjects were tested in this protocol (21 placebo [10M,10F], 13 aspirin [6M,7F], 20 ibuprofen [10M,10F]). *Nighttime/Within subjects study.* This experiment used only ibuprofen, given results from the between subjects study suggesting that ibuprofen had a larger effect than aspirin (along with data showing significant disruption of sleep after ibuprofen administration; 12). A total of 11 subjects were tested in this protocol (placebo/ibuprofen [10M,1F]). The sessions were separated by a minimum 3-day washout period and a maximum of 7 days, and drug or placebo was administered in a counterbalanced order. *Daytime/between subjects study.* Another 17 subjects (9 placebo, 8 ibuprofen; all M) were tested during the daytime hours.

NSAIDs and Melatonin Study with Body Temperature Results Replication

The procedure for this study was similar to that described for the nighttime studies described above. However, the experimental period was extended until 0300 hr, tympanic temperature was assessed every 30 min, and saliva samples (approximately 1200 µl unstimulated) were collected every 60 min for purposes of radioimmunoassay of salivary melatonin levels. All subjects

(N=10; 3M,7F) participated for two sessions, separated by a minimum 3-day washout period and a maximum of 7 days. Each subject received aspirin or ibuprofen at one session, and placebo at the other session in a counterbalanced order. Saliva samples were centrifuged at 2500 rpm immediately after collection, then frozen at -20 °C until assayed.

Tympanic temperature was recorded using the Firsttemp system (Clinical Technologies, Calabasas, CA). This system averages an error of ± .056 °C; a minimum of two consecutive measurements differing by less than or equal to this error was required at each assessment. Saliva samples were assayed for melatonin concentration using a radioimmunoassay procedure (elias usa, inc., Osceola, WI).

Statistical analysis

Difference scores were used to reduce the influence of interindividual differences in absolute body temperature and melatonin levels. Difference scores were calculated as the change from baseline just prior to NSAID administration. For example, the body temperature difference score at 2400 hr is the average difference between the body temperature levels at 2400 hr and the 2300 hr values.

Statistical tests included either repeated measures ANOVA or mixed design ANOVA (SuperANOVA v.7.0) depending upon the experimental protocol. Greenhouse-Geisser corrections for repeated measures degrees of freedom were applied to all analyses given that body temperature was assessed multiple times in each experimental period within the same subject. Probability values stated include the Greenhouse-Geisser correction.

Results

NSAIDs and BT studies

Nighttime/Between subjects design. Body temperature changes from baseline at 2300 hr for each NSAID compared to placebo are shown in Figure 1. As predicted, the decrease in body temperature was attenuated for both NSAID groups relative to the placebo group. There were

large interindividual differences in body temperature changes, but the subjects in the placebo group generally showed a normal decline (> .4 ° C) in body temperature across the testing period. The average difference in body temperature at 0100 hr between the placebo group and NSAID groups was 0.11°C. A two-way ANOVA for repeated measures (Condition x Time of Night) revealed main effects for both Condition [$F(2,51)= 3.34, p <.05$] and Time of Night [$F(8,408)=62.48, p < .001$]. Pairwise comparisons confirmed that aspirin and ibuprofen did not differ from each other, but both groups differed from placebo at every temperature assessment from 2400-0100 hr. There was also an interaction between Condition and Time of Night [$F(16,408)=2.64, p < .05$], further illustrating that body temperature for the NSAID groups was attenuated across the experimental period relative to body temperature in the placebo group. *Within subjects design.* Body temperature changes from baseline at 2300 hr after NSAID administration compared to after placebo administration are shown in Figure 2. The difference between NSAID and placebo body temperature was most evident when compared within the same individual. The average difference in body temperature at 0100 hr between the placebo group and NSAID group was .190°C. A two-way ANOVA for repeated measures (Condition x Time of Night) revealed main effects for both Condition [$F(1,12)=7.88, p < .05$] and Time of Night [$F(8,80)=20.16, p < .001$; Greenhouse-Geisser $p < .001$]. Pairwise comparisons revealed that body temperature differed significantly between NSAID and placebo at every time point after drug administration. As expected, there was also an interaction between Condition and Time of Night [$F(8,80)=7.12, p < .001$]. *Daytime/between subjects design.* Administration of an NSAID had no effect on body temperature during the daytime hours as shown in Figure 3. That is, when subjects were tested during the daytime between 1500-1700 hr, body temperature was not different for the placebo versus the NSAID groups. Body temperature was relatively flat across the experimental period for all subjects, although some subjects in both groups showed a slight increase in temperature across the experimental period, as would be expected at this time of

day when temperature is nearing its circadian peak.

NSAIDs and Melatonin Study with Body Temperature Results Replication

Body Temperature. Figure 4 shows body temperature difference scores as a function of clock time after NSAID administration compared to after placebo administration. This study replicated previous findings concerning NSAID effects on body temperature as described above. All subjects exhibited the normal nighttime decrease in body temperature under the placebo condition, although some subjects showed a greater decrease in temperature from 2300-0300 hr.

NSAID treatment attenuated the nighttime decrease in body temperature. There were no differences between aspirin and ibuprofen on body temperature changes; both NSAIDs maintained body temperature relative to placebo to a similar degree. The average change in body temperature after NSAID treatment was +.028, -.006, -.050, -.118, -.134, -.151, -.213, and -.207 ° C at 2330, 2400, 2430, 0100, 0130, 0200, 0230, and 0300 hr, respectively. In comparison, the average change in body temperature after placebo was -.028, -.056, -.129, -.202, -.286, -.353, and -.403 ° C at the same times. Thus, the average difference in body temperature at 0300 hr between the placebo groups and NSAID groups was .196°C.

An initial 3-way ANOVA confirmed that there was no effect of NSAID type (aspirin versus ibuprofen). Subsequently, a 2-way ANOVA for repeated measures (Condition x Time of Night) was performed to determine (a) whether NSAIDs maintained body temperature at a higher level than placebo, and (b) whether body temperature levels within subjects changed across the night (i.e., exhibited a circadian rhythm). This analysis confirmed that body temperature differed significantly between the NSAID and placebo conditions [$F(1,9)=18.87, p < .01$]. Specific comparisons established that body temperature was significantly higher after NSAID relative to placebo at 0030 hr and every 30 min from 0130-0300 hr ($p < .05$). There was a main effect for Time of Night as well [$F(8,72)= 41.77, p < .01$], confirming that body temperature did decline across the experimental period as expected. The presence of a significant Condition x Time of

Night interaction [$F(8,72) = 2.74$, $p < .05$] revealed furthermore that body temperature did not decline to the same degree after NSAID administration as after placebo administration.

Melatonin. Changes in melatonin levels as a function of clock time for NSAIDs compared to placebo are also shown in Figure 4. All subjects exhibited the normal nighttime circadian increase in melatonin levels during the nighttime hours under the placebo condition, although in some subjects it appeared that melatonin levels peaked by 0200 hr and lower levels were observed at 0300 hr. There were the normally observed large interindividual differences in absolute melatonin levels, but intraindividual levels of melatonin between placebo and drug nights were stable at the pretreatment baseline measure (i.e., 2300 hr).

NSAID treatment suppressed melatonin levels relative to placebo treatment. As with body temperature, there was no significant difference in amount of melatonin suppression between aspirin and ibuprofen; both NSAIDs reduced melatonin levels by approximately 75% at 2400 hr. The average change in melatonin levels after NSAID administration (relative to baseline measurement; i.e., difference scores) was -10.7, 9.3, 20.8, and 28.6 pg/ml at 2400, 0100, 0200, and 0300 hr, respectively. In comparison, the average change in melatonin levels after placebo administration was 25.4, 40.0, 49.8, and 64.0 pg/ml at the same times.

An initial 3-way ANOVA confirmed that there was no effect of NSAID type (aspirin versus ibuprofen). Subsequently, a 2-way ANOVA for repeated measures (Condition x Time of Night) was performed to determine (a) whether melatonin levels after NSAID administration differed significantly from levels after placebo administration and (b) whether melatonin levels within a subject changed across the night (i.e., circadian rhythm effect). This analysis confirmed that melatonin levels differed significantly between the NSAID and placebo conditions [$F(1,9) = 18.07$, $p < .01$]. Paired-comparisons analyses established that melatonin levels were significantly lower after NSAID than after placebo at 2400, 0100, and 0200 hr ($p < .05$), but were not different at 2300 hr (baseline) or 0300 hr. As expected, there was a main effect for Time of

Night [$F(4,36) = 5.64$, $p < .01$), with melatonin levels increasing significantly every hour ($p < .05$). The Condition x Time interaction was not significant ($p > .05$), indicating that a circadian rhythm was exhibited under both the placebo and NSAID conditions, although the amplitude of the rhythm was lower after NSAID administration.

Discussion

The primary purposes of these studies were to determine whether body temperature and melatonin levels are altered by the administration of a single dose of the NSAIDs aspirin and ibuprofen in humans. The normal circadian decrease in body temperature during the nighttime hours is attenuated by the administration of aspirin or ibuprofen, but daytime body temperature is not affected by NSAID administration. In addition, it was demonstrated that melatonin is suppressed by the administration of these NSAIDs during the nighttime hours, confirming previous reports that NSAIDs can suppress melatonin synthesis in animals (22,24) and in humans (28). These results are compatible with the hypothesis that some of the behavioral changes associated with NSAID administration, including changes in sleep (14), may be due to melatonin suppression and relatively higher body temperature. The lack of effects on daytime body temperature are also compatible with this hypothesis given that melatonin levels are very low during the diurnal hours. Further support for this argument is that NSAIDs had no effect on daytime naps relative to a placebo control condition (13).

Differences in body temperature between NSAID and placebo were apparent within 30 min after NSAID administration (i.e., 2330 hr); these differences continue and increase for up to 4 hours after administration (Figure 2). NSAID suppression of melatonin synthesis is apparent within 1 hr of administration, but in contrast to NSAID effects on body temperature, the difference in melatonin levels between NSAID and placebo become smaller across the next 4 hr (Figure 2). This time course for initial body temperature changes and melatonin suppression is in accord with evidence that prostaglandin inhibition in the peripheral nervous system begins almost

immediately after administration of an NSAID (9). The fact that there was not a one-to-one correspondence between body temperature and melatonin changes throughout the experimental period indicates that neither prostaglandin suppression nor melatonin suppression are sole determinants of body temperature changes after NSAID administration. It is unknown whether the time course for prostaglandin inhibition in the brain is similar to that for peripheral prostaglandin inhibition, or whether there are specific locations in the central nervous system which are differentially affected by NSAIDs (e.g., anterior hypothalamus versus pineal gland). It may be that a combination of prostaglandin and melatonin suppression by NSAID resulted in the observed pattern of body temperature changes in this experiment. An alternate hypothesis is that the abrupt suppression of melatonin synthesis (i.e., within 1 hr) after NSAID administration in effect signaled the circadian timing mechanism, presumably at the level of the suprachiasmatic nucluei, that it was "no longer nighttime" , and body temperature subsequently resembled a more diurnal pattern. This speculation cannot be confirmed by the present study, but it is in accord with theories suggesting that the role of endogenous melatonin is as a chemical signal of time (e.g., 2,8,20-22). Further investigations of the physiological effects of NSAIDs on prostaglandin synthesis in specific brain areas should yield an explanation of the body temperature changes observed with NSAID administration during the nighttime hours.

Assessment of melatonin levels for a longer period after NSAID administration may have determined that melatonin levels become similar for NSAID and placebo groups prior to termination of the nighttime melatonin period or even rebound beyond normal levels. NSAID administration may have simply delayed the onset of melatonin rhythm, essentially shifting the rhythm to a later clock time. Further study is needed to adequately test these hypotheses.

There were large interindividual differences in responsivity to the drug as manifested by the extent of change in melatonin and body temperature levels across subjects. This individual responsivity was evident especially in subjects who received both NSAID and placebo on

separate occasions. The basis for such individual responsivity is not understood, but may include the influence of genetic variability and gender-related factors (19) or absorption and excretion rates (18).

The present study indicates that NSAIDs can significantly alter both melatonin and body temperature during the nighttime hours. The circadian rhythms of melatonin and body temperature are thought to be primary markers of the output of the circadian system. Research in circadian rhythms has shown that by attenuating the amplitude of melatonin and body temperature (for example, with exposure to bright light), phase shifting and re-entrainment of circadian rhythms can be achieved relatively easily (7) as well as acute enhancement of nighttime performance and alertness levels (1). In relation to the present study, the implication is that judicious administration of NSAIDs might be used to facilitate phase shifts of human circadian rhythms by a means which is more convenient than bright light exposure. Beneficial effects of such phase shifting and re-entrainment of circadian rhythms could include alleviation of symptoms of jet lag, seasonal depression, and other circadian rhythm disorders.

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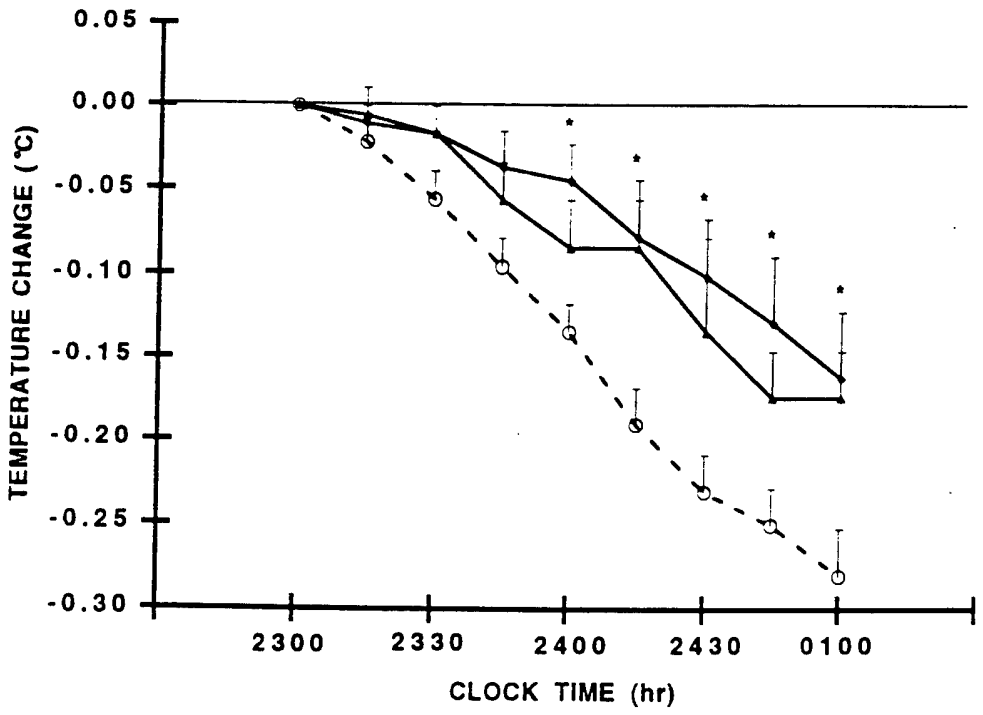
FIG. 1. Body temperature change scores (± SEM), calculated as difference from baseline measurement at 2300 hr for placebo (o---o), aspirin (Δ——Δ), and ibuprofen (o——o). * denotes different from placebo, $p < .05$. A total of 54 subjects were tested on one occasion from 2300-0100 hr. Each subject received 650 mg aspirin, 400 mg ibuprofen, or placebo.

FIG. 2. Body temperature change scores (±SEM), calculated as difference from baseline measurement at 2300 hr, for placebo (o---o) and the NSAID ibuprofen (o——o). * denotes different from placebo, $p < .05$. A total of 11 subjects were tested on two occasions from 2300-0100 hr. Each subject received 400 mg ibuprofen at one session, and placebo at the other, in a counterbalanced order.

FIG. 3. Body temperature change scores (±SEM), calculated as difference from baseline at 1500 hr for placebo (o---o) and ibuprofen (o——o). A total of 17 subjects were tested on one occasion from 1500-1700 hr. Each subject received 400 mg ibuprofen or placebo.

FIG. 4. Body temperature and melatonin change scores (±SEM), calculated as difference from baseline at 2300 hr for placebo (o---o) and ibuprofen (o——o). + denotes body temperature different from placebo, $p < .05$. * denotes melatonin different from placebo, $p < .05$. A total of 10 subjects were tested on two occasions from 2300-0300 hr. Each subject received 650 mg or 400 mg ibuprofen at one session, and placebo at the other, in a counterbalanced order.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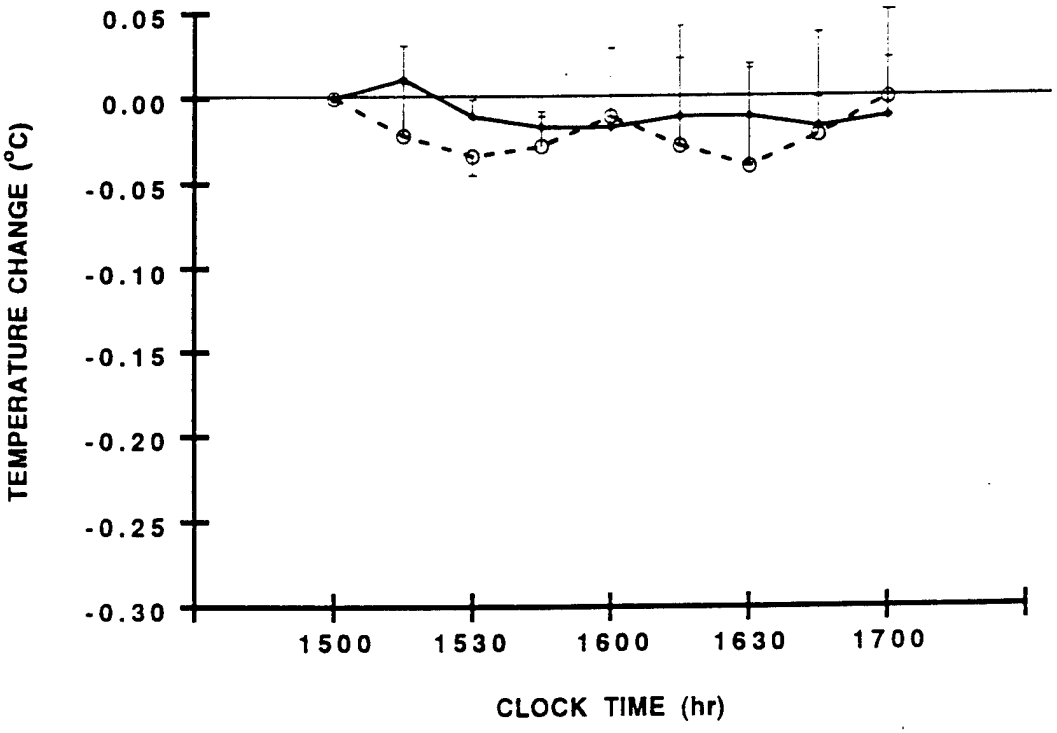
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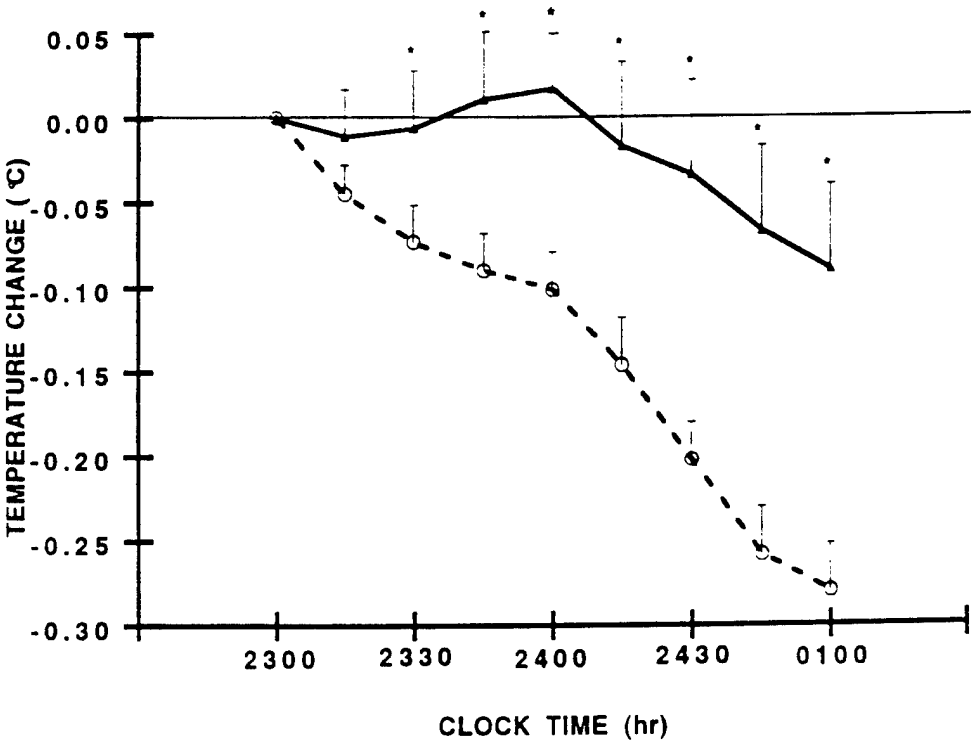
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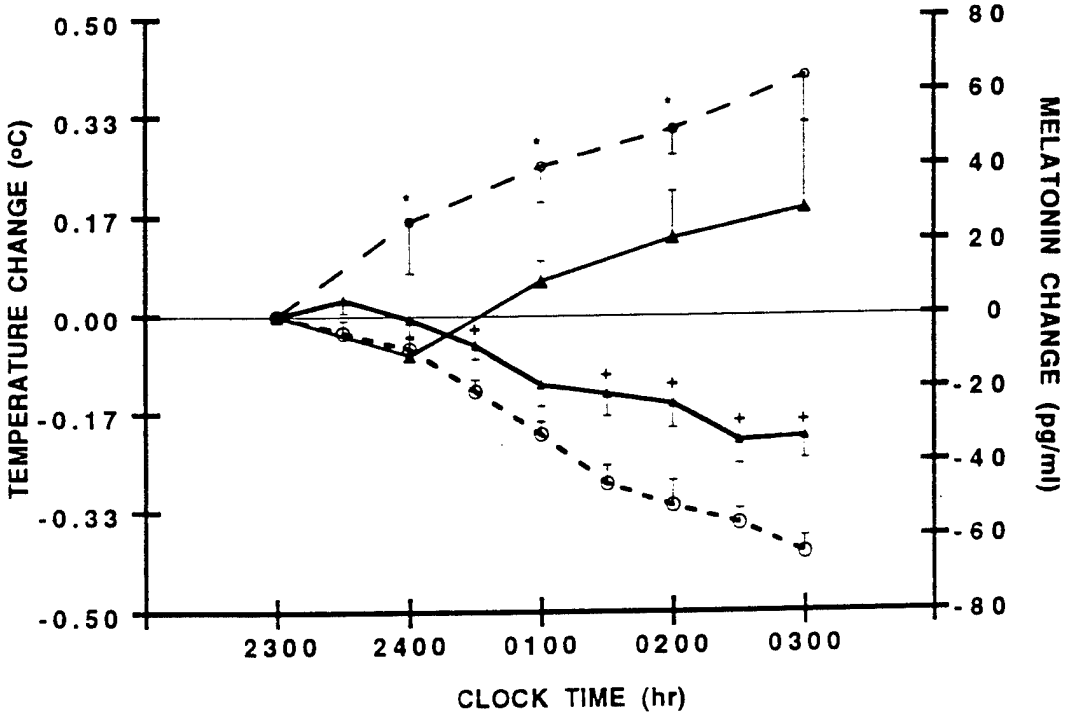
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NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND MELATONIN LEVELS IN HUMANS

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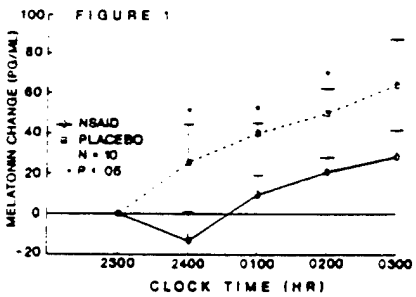
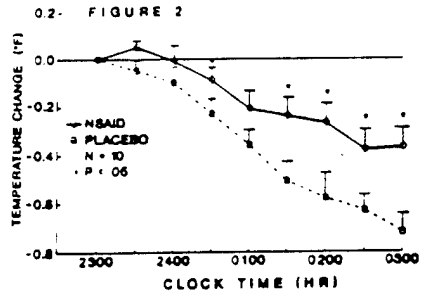
Previously we reported that nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin and ibuprofen, attenuate the nighttime decrease in body temperature (BT)[1,2] and disrupt sleep in humans[3,4]. We hypothesized that suppression of melatonin (MT) synthesis was the mechanism by which NSAIDs affect BT and sleep. Other researchers have demonstrated MT suppression by NSAIDs, but these studies tested only indomethacin or ibuprofen[5]. The present study assessed the effects of the commonly used NSAIDs aspirin and ibuprofen on nighttime MT synthesis in humans. Suppression of nighttime MT synthesis by NSAIDs may be mediated by inhibition of prostaglandin (PG) production. PGs influence MT synthesis by increasing the sensitivity of β-adrenergic receptors in the pineal gland to norepinephrine[6]. Thus, we hypothesized that inhibiting the production of PGs by administration of NSAIDs would reduce nighttime MT synthesis and attenuate the nighttime decrease in BT. Method. Subjects (N = 10; 6F, 4M; 20.2 yr) participated two nights separated by one week. The possible masking effects of various factors on BT were removed by having subjects remain seated with restricted activity for 2 hr prior to the beginning of and throughout the experiment (2100-0300 hr). At 2300 hr, 650 mg aspirin, 400 mg ibuprofen, or a placebo was administered in a double-blind manner. Tympanic temperature was recorded every 30 min and 1000 μl of saliva was collected every 60 min from 2300-0300 hr. Saliva samples were assayed for MT content using an RIA procedure (elias usa, Inc., Osceola, WI). Results. MT levels were significantly reduced compared to placebo when subjects received either NSAID. The average change in MT after NSAID (relative to baseline measurement) was -10.7, -9.3, 20.8, and 28.6 pg/ml at 2400, 0100, 0200, and 0300 hr, respectively, compared with average changes in MT after placebo of 25.4, 40.0, 49.8, and 64.0 pg/ml at the same times. Figure 1 shows changes in MT levels as a function of clock time for NSAIDs compared to placebo. An ANOVA (Condition x Time) revealed that MT levels differed significantly between the NSAID and placebo conditions [$F(1,9)=18.07$, $p<.01$]. Specific comparisons established that MT levels were significantly lower after NSAID than after placebo at 2400, 0100, and 0200 hr ($p<.05$). The Condition x Time interaction was not significant. In addition, BT was higher relative tp placebo when subjects received either NSAID. The average change in BT after NSAID (relative to baseline measurement) was .05, -.01, -.09, -.21, -.24, -.27, -.38, and -.37 °F at 2300, 2400, 2430, 0100, 0130, 0200, 0230, and 0300 hr, respectively. In comparison, the average change in BT after placebo was -.05, -.10, -.23, -.36, -.51, -.58, -.63, and -.72 °F at the same times. Figure 2 shows changes in BT as a function of clock time for NSAIDs compared to placebo. An ANOVA (Condition x Time) revealed that BT differed significantly between NSAID and placebo conditions [$F(1,9)=18.87$, $p<.01$]. Specific comparisons established that BT was significantly higher after NSAID than after placebo at 0030 hr, and every 30 min from 0130-0300 hr ($p<.05$).





Conclusions. The NSAIDs aspirin and ibuprofen suppress nighttime MT synthesis and attenuate the nighttime decrease in BT in humans. The changes in MT and BT did not show a one-to-one relationship. The lack of a strong relationship between MT suppression and changes in BT may be due to (a) other factors playing a role in the nighttime decrease in BT or (b) assay insensitivity to the levels of MT involved. It is likely, however, that the changes in BT after NSAID administration are partially mediated by alterations in MT synthesis given that both correlational and empirical evidence support the role of MT as a thermoregulatory agent[2,7,8,9]. The present data are compatible with our previous findings showing that low doses of NSAIDs have immediate effects on BT and on sleep during the nighttime hours[1-3]. In addition, these data are compatible with our hypothesis that MT is involved in thermoregulation. The hormone has hypothermic effects, and thus by suppressing MT synthesis, the nighttime decrease in BT is attenuated[1,2,7,8,9]. The mechanisms by which NSAIDs suppress MT may be related to the fact that NSAIDs inhibit PG production, and PGs can increase MT synthesis[5].

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IMMEDIATE EFFECTS OF PHOTIC STIMULATION ON MELATONIN AND TEMPERATURE ACROSS CONSECUTIVE NIGHTS[1]

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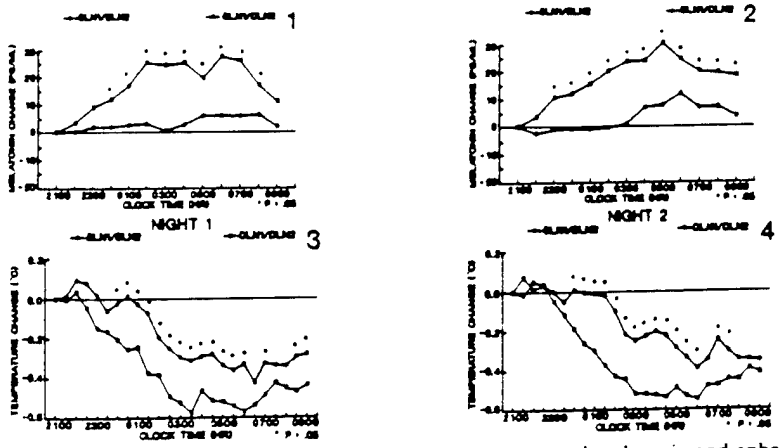
Exposure to photic stimulation during the nighttime has immediate and delayed effects on melatonin and temperature[2,3,4]. Immediate effects occur during exposure to bright (> 500 lux) light while delayed effects occur after exposure to bright light. During 24-hr periods of sleep deprivation, nocturnal exposure to photic stimulation enhances temperature and improves alertness/performance[2,3]. These effects may be mediated by the suppression of melatonin[5]. Light-induced melatonin suppression is attainable for several cycles in nonhumans[6]; however, it is not known whether such an effect is attainable in humans. Given the nonhuman finding[6] and given the similarity in the circadian system across species[7], it was hypothesized that exposure to bright light during the nighttime would decrease melatonin and increase temperature relative to exposure to dim light and that this effect would occur on consecutive nights. ***Methods***. Twenty male college students (average age = 20 years) were deprived of sleep for 48 hr starting at 1030 hr. Throughout this 48 hr, a constant-routine procedure was used; that is, physical activity, light intensity, and food/beverage intake were controlled. Physical activity was minimized by having subjects remain seated upright throughout the testing session. Participants were randomly assigned to one of two Conditions: exposure to either bright (> 2,500 lux; BLN1/BLN2; N = 10) or dim (< 100 lux; DLN1/DLN2; N = 10) light from 2100-0900 hr on Nights 1 and 2. Subjects otherwise remained under dim light. Food/beverage intake was allowed every 3 hr. Salivary melatonin (RIA; Elias, Osceola, WI) was assessed every 60 min and tympanic temperature (IMS, Carlsbad, CA) was assessed every 30 min. Melatonin data were lost for one BLN1/BLN2 subject. ***Results***. Figures 1-4 depict the average change in melatonin or temperature as a function of Condition, Time, and Night. Difference scores were calculated with the first measurement of each



night serving as the zero point. Nocturnal exposure to bright light suppressed melatonin and enhanced temperature relative to exposure to dim light on each Night. These observations were supported by statistical analyses (ANOVA for repeated measures with Greenhouse-Geisser *df* corrections). Two-way ANOVAs (Condition x Time) indicated an effect of Condition on each Night (2100-0900 hr). On Night 1, the average melatonin changes and standard errors were 3.19(0.72) pg/ml under bright and 18.04(2.52) pg/ml under dim light ($p < .05$) while the average temperature changes and standard errors were -0.19(0.05)°C under bright and -0.37(0.06)°C under dim light ($p < .05$). On Night 2, the average melatonin changes and standard errors were 3.22(1.56) pg/ml under bright and 18.52 (2.32) pg/ml under dim light ($p < .05$) while the average temperature changes and standard errors were -0.17(0.05)°C under bright and -0.35(0.07)°C under dim light ($p < .05$). Condition x Time interactions were also noted and indicate bright and dim light had different effects at different clock times. In fact, post-hoc analyses (2-way ANOVA, Condition x Time) revealed differences at several times (Figures 1-4). Three-way ANOVA (Night x Condition x Time) indicated similar effects on each Night. It is unlikely differential physical activity in the bright and dim light groups affected the results since actigraphic data indicated no such differences in a similar experiment[3]. ***Conclusions***. Effects of photic stimulation (e.g., melatonin suppression) are evident in most species tested[7]. That similar results were obtained on consecutive nights demonstrates another similarity in the circadian system of humans and nonhumans. Nocturnal exposure to bright light improves alertness and performance[2]. Since these effects may be mediated, in part, by melatonin suppression[5] and since the present results showed suppression occurred on consecutive nights, photic stimulation may be useful for alleviating some consequences associated with extended periods of sleep deprivation[8].

[1]Supported by The Army Research Institute (MDA 903-93-K-0002) and The Ohio Board of Regents Selective Excellence Program.

[2]Badia et al. Bright light effects on body temperature, alertness, EEG and behavior. *Physiol. Behav*, 1991, *50*(3): 583-588.
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BRIGHT LIGHT AFFECTS BODY TEMPERATURE AND MELATONIN LEVELS DURING 48 HOURS OF SLEEP DEPRIVATION[1]

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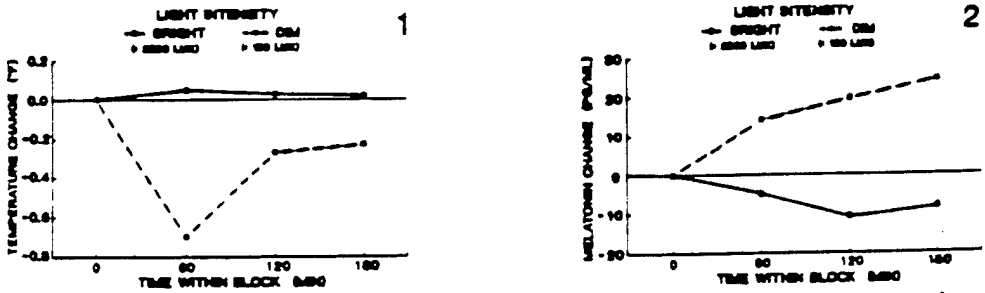
Earlier we showed that photic stimulation (≥ 5,000 lux) had immediate effects during the nighttime hours on alertness, body temperature, EEG, and performance during 24 hr of sleep deprivation[2]. Because these effects occurred only during the nighttime when melatonin is synthesized and released and with light intensities known to suppress melatonin, we assumed that melatonin was also suppressed. Additional research conducted in our laboratory and the work of others suggests that melatonin mediates the physiological and psychological effects of photic stimulation[3]. In the present investigation, we tested whether bright light (≥ 2,500 lux) would continue to enhance temperature and suppress melatonin during 48 hr of sleep deprivation. *Method.* Six male college students (average age = 20 years) were tested. Participants arrived at 2200 hr and slept from 0000 to 0900 hr prior to beginning a 48-hr period of sleep deprivation. Throughout this period, a modified constant routine procedure was implemented. That is, physical activity, light exposure, and food/beverage intake were rigorously controlled. Physical activity was minimized by having the subjects remain seated upright throughout the testing session. During the daytime (0900-2100 hr) subjects remained under dim light (≤ 100 lux) while during the nighttime (2100-0900 hr) subjects remained under either dim light (≤ 100 lux) or bright light (≥ 2500 lux) administered in alternating and counterbalanced 3 hr blocks. Food and beverages were allowed only every 3 hr in similar aliquots. The primary dependent measures of the study were tympanic temperature (Intelligent Medical Systems, Carlsbad, CA) which was recorded every 60 min and salivary melatonin (RIA; Elias USA, Osceola, WI) which was sampled every 60 min. To determine if there were any differences in physical activity under the two light intensities, the participants wore an actigraph (Ambulatory Monitoring, Ardsley, NY) on their nondominant wrist. *Results.* Nocturnal exposure to bright light significantly enhanced temperature and significantly suppressed melatonin relative to dim light. This effect occurred in a similar manner on both nights. We have reported the effects of bright light during 24 hr of sleep deprivation[4]; therefore, only the results of the second 24 hr will be described. The mean nocturnal temperature level for the second night of deprivation (2100-0900 hr) was 98.8 °F under bright light and 98.6 °F under dim light ($p < .05$). The mean nocturnal melatonin level was 12.32 pg/ml under bright light and 28.35 pg/ml under dim light ($p < .05$). The accompanying figures depict the average change in temperature (Figure 1) or melatonin (Figure 2) as a function of light intensity and time within block for the entire second night (2100-0900 hr). Difference scores were calculated with the first



measurement of each 3 hr block serving as the zero point. This initial measurement was then subtracted from each subsequent measurement within the block. The figures illustrate an advantage of measuring melatonin as masking effects are evident in the temperature data[4]. An additional finding was that dim light melatonin levels increased across the deprivation period. Average dim light melatonin levels were 21.91 pg/ml during the later portion of the first 24 hr (0300-0900 hr) and 43.31 pg/ml during the later portion of the second 24 hr ($p < .05$). The actigraphic data indicated that there were no differences in physical activity under the two light intensities. *Discussion.* Temperature and melatonin levels were inversely related during the deprivation period. That is, exposure to bright light was associated with an increase in temperature and a decrease in melatonin (relative to dim light) whereas exposure to dim light was associated with a decrease in temperature and an increase in melatonin (relative to bright light). These results are consistent with our previous research[4] which utilized a shorter period of sleep deprivation and it provides additional support for the notion that melatonin plays an important role in thermoregulation[3]. It is interesting to speculate about the finding concerning the increase in dim light melatonin levels across the deprivation period. This result may represent the likely phase delay induced by the photic stimulation; but, this possibility seems improbable since the melatonin levels obtained on the second night were considerably higher than those obtained on the first night. A more likely possibility is that this finding represents a compensatory increase in melatonin levels resulting from suppression during the first 24 hr. While it is unlikely that such a rebound was induced by the sleep deprivation alone[5], it may be that a rebound was induced by the combination of sleep deprivation and photic stimulation[6]. We are testing this hypothesis.

[1]This research was supported by The Army Research Institute, Contract MDA 903-93-K-0002.

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CIRCADIAN EFFECTS OF PHOTIC STIMULATION ON THE MELATONIN AND TEMPERATURE RHYTHMS IN HUMANS[1]

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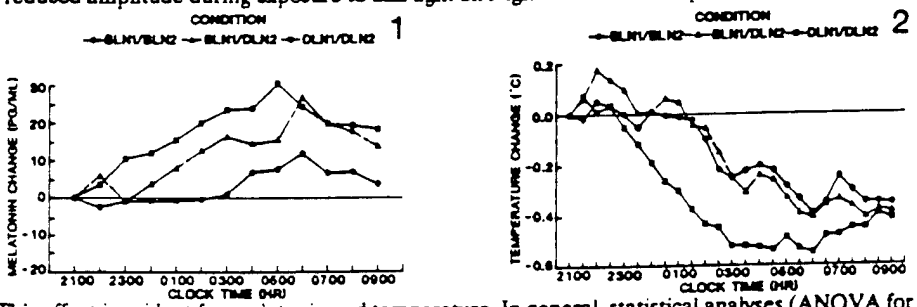
During nighttime exposure to bright (> 500 lux) light, melatonin is suppressed, temperature is enhanced, and alertness/performance is improved. The relationship between such immediate effects occurring during light exposure[2] and the delayed effects occurring subsequent to light exposure[3,4,5] of photic stimulation is unclear. Immediate effects of nocturnal exposure to bright light may produce unwanted delayed effects such as changes in amplitude. The magnitude and direction of these changes are unclear, however. Some data suggest that exposure to bright light during the nighttime produces a subsequent decrease in temperature amplitude[3,4,5]. However, other data suggest that light-induced suppression of melatonin during the nighttime produces a subsequent and compensatory increase in its production and therefore an increase in melatonin amplitude[6]. These conflicting findings may have resulted from the use of different circadian markers (temperature, melatonin). Therefore, this study tested the effects of nocturnal exposure to bright light on the amplitude of the melatonin and temperature rhythms. ***Methods***. Thirty male college students (average age = 20 years) were deprived of sleep for 48 hr starting at 1030 hr. Throughout the 48 hr, a constant-routine procedure was implemented; that is, physical activity, light intensity, and food/beverage intake were controlled. Physical activity was minimized by having subjects remain seated upright throughout the testing session. Participants were randomly assigned to one of three Conditions: (a) bright (> 2,500 lux) light on Nights 1 and 2 from 2100-0900 hr (BLN1/BLN2; N = 10); (b) bright light on Night 1 from 2100-0900 hr and dim (< 100 lux) light on Night 2 from 2100-0900 hr (BLN1/DLN2; N = 10); or (c) dim light on Nights 1 and 2 from 2100-0900 hr (DLN1/DLN2; N = 10). Subjects otherwise remained under dim light. Food/beverage intake was allowed every 3 hr. Salivary melatonin (RIA; Elias, Osceola, WI) was assessed every 60 min and tympanic temperature (IMS, Carlsbad, CA) every 30 min. Melatonin data were lost for one BLN1/BLN2 and one BLN1/DLN2 subject. ***Results***. Difference scores were calculated with the first measurement of each night serving as the zero point. Exposure to bright light decreased melatonin and increased temperature relative to exposure to dim light on each Night. Night 1 data were similar to those reported[2]; therefore, only the Night 2 data are presented. Figures 1 and 2 depict the average change in melatonin or temperature as a function of Condition and Time. Exposure to bright light on Night 1 reduced amplitude during exposure to dim light on Night 2 relative to exposure to dim light on both



nights. This effect is evident for melatonin and temperature. In general, statistical analyses (ANOVA for repeated measures with Greenhouse-Geisser *df* corrections) supported these observations. Two-way ANOVAs (Condition x Time) indicated melatonin and temperature differences among the three groups. During Night 2, the average melatonin changes and their standard errors were 3.22(1.56), 12.79(2.59), and 18.52(2.52) pg/ml in the BLN1/BLN2, BLN1/DLN2, and DLN1/DLN2 groups, respectively (p < .05) while the average temperature changes and their standard errors were -0.17(0.05), -0.17(0.07), and -0.35(0.07) °C in the BLN1/BLN2, BLN1/DLN2, and DLN1/DLN2 groups, respectively (p < .05). Post-hoc analyses of the melatonin data revealed the BLN1/BLN2 and DLN1/DLN2 groups were different but the BLN1/DLN2 and DLN1/DLN2 groups were not. Comparable analyses of the temperature data revealed effects similar to those of Night 1 but in the absence of bright light; that is, the BLN1/BLN2 and BLN1/DLN2 groups were different from the DLN1/DLN2 group but not from each other. ***Conclusions***. Statistical analyses indicated nocturnal exposure to bright light on Night 1 did not suppress melatonin amplitude during exposure to dim light on Night 2; however, visual analysis suggests suppression. Perhaps statistical power was not sufficient and/or variability too large to detect suppression. Clearly, there was no evidence of a melatonin rebound. Nocturnal exposure to bright light did suppress temperature amplitude, however. Differential melatonin and temperature results are surprising since both rhythms are reliable markers of the circadian system[4,7] and melatonin is a regulator of temperature[7]. Given this latter finding and given that amplitude typically recovers within 24-hr[4], it is likely melatonin amplitude is returning to its normal level more rapidly than temperature amplitude. This notion is supported by a similar finding[3]. In sum, amplitude is attenuated following all-night exposure to bright light and such an effect should be considered when using photic stimulation to enhance alertness and performance during the nighttime hours.

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Reliability of Salivary Melatonin

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Melatonin is thought to be involved in the regulation of many systems due to its ubiquitous biological effects. It is also a primary marker of the circadian system. Melatonin (MT) can be measured in saliva and serum but due to saliva's ease of collection it may be the preferred method. Given the latter, it is important that the reliability of salivary MT within individuals across multiple nights be assessed. Previous research indicates that the nightly MT rhythm in saliva is highly correlated with MT levels in blood and that salivary MT levels are approximately 30% of blood MT levels[1,2]. Plasma[3,4] and serum[5] MT levels have shown high reliability across repeated testing sessions but saliva MT reliability is less well established. Therefore, we investigated the reliability of the salivary MT profile across successive weeks. **Methods**. Seven subjects (6 males, 1 female, age range 20-26 years) participated on 3 evenings, with each successive testing session separated by at least 1 week but not more than 2 weeks. Subjects adhered to the following restrictions prior to participation: no medications of any kind for 72 hrs; no alcohol for 24 hrs; no caffeine for 6 hrs; and no food for 2 hrs. Subjects reported to the laboratory at 1930 hr at which time they completed consent and medical history forms. They were seated in comfortable chairs and movement was restricted. Light levels were ≤ 100 lux at all times. From 2000 - 0200 hr, hourly 2 ml saliva samples were collected into polystyrene tubes. Samples were then centrifuged at 2500 rpm and frozen at -20°C until assayed. Saliva samples were packed in dry ice and shipped overnight air mail to Elias USA (Osceola, WI) for radioimmunoassay. **Results**. MT levels were quite similar across days for most subjects. Similar to previous reports, intra-individual variation was low while inter-individual variation was substantial. Figure 1 shows individual subject MT profiles for all 3 nights. Individual subjects showed a range of correlations across the 3 nights from $r = .08$ (S 05, N2 vs N3) to $r = .98$ (S 01, N1 vs N2). The average across all subjects was: N1 vs N2: $r = .98$ ($p<.001$), N1 vs N3: $r = .97$ ($p<.001$), N2 vs N3: $r = .98$ ($p<.001$). **Discussion**. Salivary MT levels were reliable across time in the present study at levels lower than serum as previously reported. MT levels in saliva appear to be a reliable marker similar to serum MT estimation. These results are consistent with the hypothesis that the circadian system is relatively stable across time. Some have argued that the circadian system is more variable than previously thought[7]. While the present study did not employ a strict constant routine, under controlled conditions MT onset and subsequent MT levels for individual subjects remained reliably consistent across repeated sessions. These results suggest that saliva samples may be adequate for both amplitude and phase determination. The present results support MT as a reliable biological marker regardless of the procedure used to detect it, i.e., blood, urine or saliva.

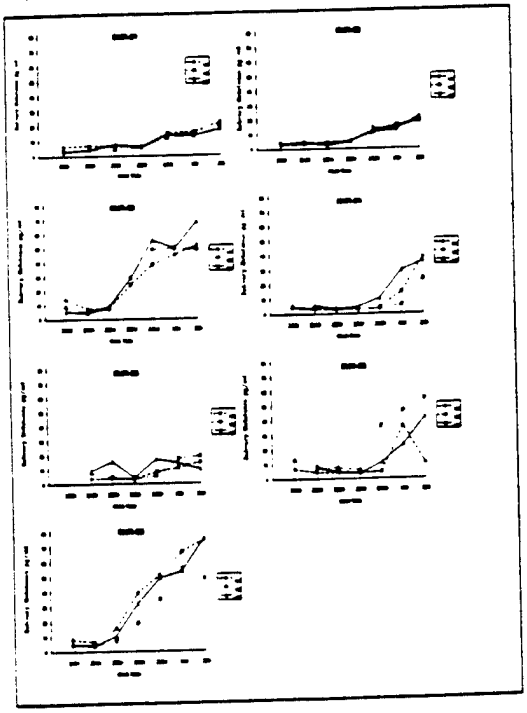

Figure 1- Individual subject salivary MT profiles.

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EFFECTS OF CAFFEINE, BRIGHT LIGHT, AND THEIR COMBINATION ON NIGHTTIME MELATONIN AND TEMPERATURE DURING TWO NIGHTS OF SLEEP DEPRIVATION

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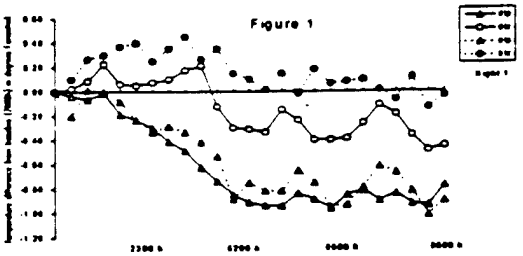
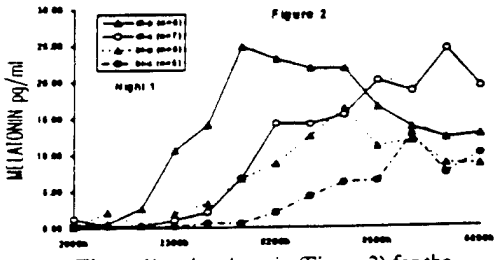
While noradrenergic receptor stimulation is apparently the major control of melatonin biosynthesis in the pineal, other molecules have also been shown to effect pineal activity. Included in the list is the neuromodulator adenosine. Adenosine and adenosine analogs increase melatonin synthesis in the pineal for both in vivo and in vitro preparations in the rat[1]. In general, adenosine injections increase melatonin synthesis two to three fold compared to saline controls. Adenosine is thought to exert its actions on melatonin through adenosine A_{2b} receptors on pinealocytes which may mimic or effect the action of alpha adrenergic receptors on the pineal[2]. A circadian rhythm in pineal adenosine exists with peak levels occurring at a similar time to when melatonin levels increase[3]. Several substances can antagonize the effects of adenosine, one of which is caffeine. Studies suggest that the stimulatory effects of caffeine are due to the blockade of adenosine receptors[4]. Therefore, since caffeine antagonizes the effects of adenosine, and adenosine is involved in melatonin synthesis, caffeine may have an effect on nighttime melatonin production. The present study assessed the latter. We also assessed whether exposure to bright light over a 48 hour sleep deprivation period affected melatonin and temperature and whether the combination of bright light and caffeine had a greater effect than either alone. Subjects. Twenty-five male college students (Mean age 19.32 yr) were tested. Participants arrived at the lab at 2000 hr and slept from 0030 to 1030 hr prior to beginning a 48-h period of sleep deprivation. Throughout this period, a modified constant routine procedure was implemented and physical activity, light exposure, and food intake were rigorously controlled. During the day subjects remained under dim light (≤ 100 lux) while during the night (2000-0800 hr) subjects remained under either dim (≤ 100 lux) or bright light (≥ 2,500 lux). Half the subjects in each lighting condition received either placebo (sugar pill) or 200 mg of caffeine with water and food at 2000 and 0200h both nights. Caffeine and placebo were administered in a double blind manor. In sum, four treatment conditions were tested: Dim light-Placebo (dlp); Dim light-Caffeine (dlc); Bright light-Placebo (blp); Bright light-Caffeine (blc). Aliquots of food and beverages were given every three hr. Salivary melatonin (RIA; Elias USA, Osceola, WI) was sampled every 60 min at night, while tympanic temperature was recorded every 60 min during the day and every 30 min at night. Results. Both caffeine administration and bright photic stimulation decreased melatonin synthesis and attenuated the nocturnal decrease in tympanic temperature across time. In general, bright light tended to decrease melatonin relative to dim light and caffeine tended to decrease melatonin relative to placebo. However, the combined bright light-caffeine condition showed the largest effects on suppressing melatonin and attenuating the drop in nighttime temperature, i.e., melatonin was lowest and temperature highest for the combined condition. These effects were observed on both nights across a 48





hr period. The accompanying figures depict the change in temperature (Figure 1) and melatonin (Figure 2) for the different treatment conditions for Night 1. Both caffeine and bright light appear to delay the melatonin rhythm and maintain temperature at higher levels compared to dim light-placebo (with the exception of bright light having little effect on temperature on Night 1 - effects were observed on Night 2). Significant group by time interactions were observed for melatonin ($p < .01$) and temperature ($p < .01$) data on both nights. Discussion. A number of pharmacological agents affect endogenous melatonin levels (e.g., antipsychotics, anxiolytics, hypnotics, tryptophan, beta blockers, alcohol, NSAIDS). The present results extend the list to include caffeine. Caffeine should thus be considered a masking agent affecting both temperature and melatonin and should be controlled in circadian rhythm studies. The present data show that caffeine has an immediate effect on melatonin and temperature but whether delayed circadian effects occur can not be determined from the design used. The finding that caffeine effects nighttime melatonin and temperature has implications for nighttime performance, phase shifting and sleep.

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THE COMBINED EFFECTS OF BRIGHT LIGHT AND CAFFEINE ON NIGHTTIME ALERTNESS AND PERFORMANCE DURING TWO NIGHTS OF SLEEP DEPRIVATION

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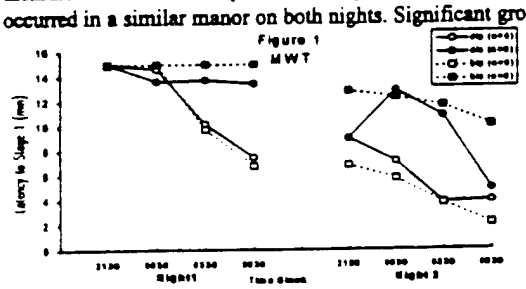
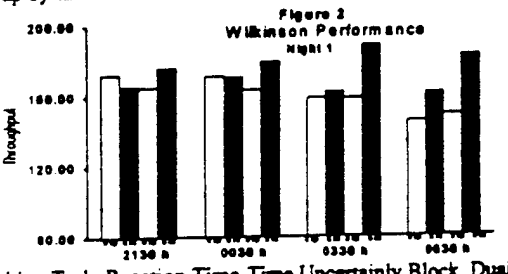
Maintaining nighttime alertness and high levels of performance are vital for some occupations, especially for individuals working out of phase with their circadian rhythms, i.e., working when they normally would be sleeping. Among others, the latter would include airline pilots, truck drivers, military operations personnel, workers in nuclear power plants, air traffic controllers and shift workers in general. Both caffeine[1,2] and bright light[3] appear to be effective treatments for combating degradations in alertness and performance; especially during the early morning hours. The efficacy of the two treatments in maintaining or enhancing alertness during the nighttime hours has not been compared. The present study does this using treatment levels previously shown to be effective. In addition, we assessed whether the combined use of caffeine and bright light had a greater effect on alertness and performance than either treatment alone. Subjects. Twenty-eight male college students (Mean age 19.33 yr) were tested. Participants arrived at the lab at 2000 hr and slept from 0030 to 1030 hr prior to beginning a 48-h period of sleep deprivation. Throughout this period, a modified constant routine procedure was implemented and physical activity, light exposure, and food intake were controlled. During the day subjects remained under dim light (≤ 100 lux) while at night (2000-0800 hr) subjects remained under either dim (≤ 100 lux) or bright light (≥ 2,500 lux). Half the subjects in each lighting condition received either placebo (sugar pill) or 200 mg of caffeine with water and food at 2000 and 0200h both nights. Caffeine and placebo were administered in a double blind manor. In sum, four treatment conditions were tested: Dim light-Placebo (dlp); Dim light-Caffeine (dlc); Bright light-Placebo (blp); Bright light-Caffeine (blc). Performance Tasks. A variety of tasks developed by our laboratory and by others[4,5] and also those found on the Walter Reed PAB and UTCPAB were used. A Maintenance of Wakefulness Test (MWT) was also administered to test for alertness level. Prior to testing, subjects had sufficient exposure to tasks in order to minimize practice effects. Performance and alertness were assessed every three hours from 2000 to 0800 hr each night. Results. Caffeine ingestion appears more effective in enhancing nighttime alertness and performance than exposure to bright photic stimulation. In general, bright light tended to increase performance on some tasks relative to dim light (see also[6])whereas caffeine increased alertness (MWT) and performance relative to no caffeine on most tasks. Bright light did not enhance alertness relative to dim light. However, the combined bright light-caffeine condition showed the largest enhancing effects on the alertness and performance measures. In fact for every task, the bright-light caffeine combination produced the best performance. These effects occurred in a similar manor on both nights. Significant group by time interactions for throughput measures were





observed for a number of tasks across the night (e.g., Switching Task, Reaction Time-Time Uncertainly Block, Dual Task, Wilkinson Four Choice Reaction Time; all $p < .01$). The accompanying figures depict examples of alertness (Figure 1-Nights 1& 2) and performance (Figure 2-Night 1) for the different treatment conditions. Discussion. Caffeine given in two moderate doses at 2000 and 0200h was able to enhance alertness and performance across two nights of sleep deprivation. This finding is contrary to previous reports suggesting that caffeine has little effect on performance on Night 2 of sleep deprivation[7].The difference among the studies could be due to the timing of caffeine administration. In the present study caffeine was administered at times which were thought optimally to affect body temperature and melatonin levels. In part caffeine's ability to enhance nighttime alertness and performance may be related to its affects on melatonin and temperature[8]. The finding that the combination of bright light and caffeine produced the largest effects in enhancing nighttime alertness and performance suggests the treatments have additive effects.

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This research was supported by the Army Research Institute, Contract MDA 903-93-K-0002 and also from an Ohio Board of Regents Selective Excellence Program

Figure Captions

Figure 1. Melatonin change (pg/mL) as a function of clock time (hr) for Night 1. The BLN1/BLN2 group is represented by the light line with circles, the BLN1/DLN2 group is represented by the dashed line with triangles, and the DLN1/DLN2 group is represented by the heavy line with squares. The BLN1/DLN2 group differs from the DLN1/DLN2 group ($p < .05$), the BLN1/BLN2 group differs from the DLN1/DLN2 group ($p < .05$), but the BLN1/DLN2 group does not differ from the BLN1/BLN2 group ($p > .05$).

Figure 2. Temperature change (°C) as a function of clock time (hr) for Night 1. The BLN1/BLN2 group is represented by the light line with circles. The BLN1/DLN2 group is represented by the dashed line with triangles and the DLN1/DLN2 group is represented by the heavy line with squares. The BLN1/DLN2 group differs from the DLN1/DLN2 group ($p < .05$), the BLN1/BLN2 group differs from DLN1/DLN2 group ($p < .05$), but the BLN1/DLN2 group does not differ from BLN1/BLN2 group ($p > .05$).

Figure 3. Melatonin change (pg/mL) as a function of clock time (hr) for Night 2 The BLN1/BLN2 group is represented by the light line with circles and the DLN1/DLN2 group is represented by the heavy line with squares. Asterisks indicate the clock times at which significant differences ($p < .05$) between the two groups were obtained.

Figure 4. Temperature change (°C) as a function of clock time (hr) for Night 2 The BLN1/BLN2 group is represented by the light line with circles and the DLN1/DLN2 group is represented by the heavy line with squares. Asterisks indicate the clock times at which significant differences ($p < .05$) between the two groups were obtained.

Figure 5. Top: Melatonin change (pg/mL) as a function of clock time (hr) for Night 1. The placebo group is represented by a dotted line with triangles, the bright light group is represented by a dashed line with circles, and the NSAID group is represented by a solid line with squares. == indicates the clock times at which significant differences ($p < .05$) between the NSAID and placebo groups were obtained, + indicates the clock times at which significant differences ($p < .05$) between the bright light and placebo groups were obtained, and * indicates the clock times at which significant differences ($p < .05$) between the NSAID and bright light groups were obtained. Bottom: Melatonin change (pg/mL) as a function of clock time (hr) for Night 2 plotted in a similar manner as Night 1.

Figure 6. Top: Temperature change (C) as a function of clock time (hr) for Night 1. The placebo group is represented by a dotted line with triangles, the bright light group is represented by a dashed line with circles, and the NSAID group is represented by a solid line with squares. + indicates the clock times at which significant differences ($p < .05$) between the bright light and placebo groups were obtained and * indicates the clock times at which significant differences ($p < .05$) between the NSAID and bright light groups were obtained. Bottom: Temperature change (C) as a function of clock time (hr) for Night 2 plotted in a similar manner as Night 1.

Figure 7. Melatonin change (pg/mL) as a function of clock time (hr) for Night 2. The BLN1/BLN2 group is represented by the light line with circles. The BLN1/DLN2 group is represented by the dashed line with triangles and the DLN1/DLN2 group is represented by the heavy line with squares. The BLN1/DLN2 group does not differ from the DLN1/DLN2 group ($p > .05$), the BLN1/DLN2 group does not differ from the BLN1/BLN2 group ($p > .05$), but. the BLN1/BLN2 group differs from the DLN1/DLN2 group ($p < .05$).

Figure 8. Temperature change (°C) as a function of clock time (hr) for Night 2. The BLN1/BLN2 group is represented by the light line with circles, the BLN1/DLN2 group is represented by the dashed line with triangles, and the DLN1/DLN2 group is represented by the heavy line with squares. The BLN1/DLN2 group differs from the DLN1/DLN2 group ($p < .05$), the BLN1/BLN2 group differs from the DLN1/DLN2 group ($p < .05$), but the BLN1/DLN2 group does not differ from BLN1/BLN2 ($p > .05$).

Figure 9. Twelve hour salivary melatonin levels (Log [x+1]) for dim-light placebo (DLP), dim-light caffeine (DLC), bright-light placebo (BLP) and bright-light caffeine (BLC) on both nights of sleep deprivation. Melatonin levels are lower under caffeine and bright light treatments compared to dim-light placebo. The combined treatment of bright light and caffeine resulted in the lowest melatonin levels both nights.

Figure 10. Twelve hour tympanic temperature data (difference from 2000 hr baseline) for dim-light placebo (DLP), dim-light caffeine (DLC), bright-light placebo (BLP) and bright-light caffeine (BLC) on both nights of sleep deprivation. Tympanic temperature is higher under caffeine and bright light treatments compared to dim-light placebo. The combined treatment of bright light and caffeine resulted in the highest temperature levels both nights.

Figure 11. Forty-eight hour tympanic temperature data for dim-light placebo, dim-light caffeine, bright-light placebo and bright-light caffeine conditions during sleep deprivation. Placebo conditions exhibit a typical circadian rhythm in temperature. The dim-light caffeine condition appears to attenuate the circadian rhythm in temperature, especially on night 1. The combined treatment of bright-light and caffeine produced high temperature levels across the entire deprivation period.

Figure 12. A comparison of the inverse relationship between melatonin and temperature for dim-light placebo, bright-light placebo, dim-light caffeine and bright-light caffeine conditions during two nights of sleep deprivation. The relationship between melatonin and temperature is robust in all conditions albeit reduced by the caffeine treatments.

Figure 13. Latency to sleep on the Maintenance of Wakefulness Test during sleep deprivation every three hours nightly and once during the daytime. Caffeine treatments maintained higher levels of alertness compared to placebo treatments.

Figure 14. Subjective sleepiness on the Stanford Sleepiness Scale immediately prior to sleep deprivation (P - 1700 h) and every three hours nightly during sleep deprivation. The higher the score indicates higher sleepiness. Dim-light caffeine (DLC) on night 1 and bright-light placebo (BLP) on night 2 reduced subjective sleepiness relative to dim-light placebo (DLP). The combined treatment of bright light and caffeine (BLC) produced high subjective alertness across the entire sleep deprivation period.

Figures 15-17. Performance on tasks with a memory component during the last practice trial prior to sleep deprivation (P - 1700 h) and every three hours nightly during two nights of sleep deprivation. Caffeine treatments (DLC and BLC) showed better performance compared to dim-light placebo (DLP) most of the time. The combined treatment of bright light and caffeine showed the best overall performance. The bright light placebo treatment improved performance for few tasks with a memory component.

Figure 18. Performance on tasks without a memory component during the last practice trial prior to sleep deprivation (P - 1700 h) and every three hours nightly during two nights of sleep deprivation. Caffeine and bright light treatments (DLC, BLP and BLC) showed better performance compared to dim-light placebo (DLP) most of the time. The combined treatment of bright light and caffeine showed the best overall performance.

Figure 19. Temperature change (°F) as a function of Time within Block (Min) and light intensity (bright light, •—•, ≥ 2,500 lux; dim light, --- , ≤ 100 lux).

Figure 20. Melatonin change (pg/mL) as a function of Time within Block (Min) and light intensity (bright light, •—•, ≥ 2,500 lux; dim light, --- , ≤ 100 lux).

Figure 21. Temperature change (°F) as a function of Clock Time (hr) and light intensity (bright light, BL, ≥ 2,000 lux ; dim light, DL, ≤ 50 lux).

Figure 22. Salivary melatonin (pg/mL) as a function of Clock Time (hr) and Night (Night 1, N1, o---o; Night 2, N2, ■—■; Night 3, N3, ▲—▲) for seven individual subjects.

Figure 23. Schematic representation of experimental protocols. For studies in which only temperature was assessed, drug or placebo was administered at 1500 (daytime study) or 2300 hr (nighttime studies), and tympanic temperature was assessed every 15 min for 2 hr. For studies in which both temperature and melatonin was assessed, drug or placebo was administered at 2300 hr, tympanic temperature was assessed every 30 min, and saliva samples were collected every 60 min.

Figure 24. Mean body temperature change scores (+/- SEM), calculated as difference from baseline measurement at 2300 hr for placebo (o---o), aspirin (Δ—Δ), and ibuprofen (•—•). An asterisk (*) denotes that both aspirin and ibuprofen were significantly different ($p < .05$) from placebo. A total of 54 subjects were tested on one occasion from 2300 to 0100 hr. Each subject received either 650 mg aspirin, 400 mg ibuprofen, or placebo.

Figure 25. Mean body temperature change scores (+/- SEM), calculated as difference from baseline measurement at 2300 hr for placebo (o---o) and ibuprofen (•—•). An asterisk (*) denotes that ibuprofen was significantly different ($p < .05$) from placebo. A total of 11 subjects were tested on two occasions from 2300 to 0100 hr. Each subject received 400 mg ibuprofen on one occasion and placebo on another (in a counterbalanced order).

Figure 26. Mean body temperature change scores (+/- SEM), calculated as difference from baseline measurement at 1500 hr for placebo (o---o) and ibuprofen (•—•). A total of 17 subjects were tested on one occasion from 1500 to 1700 hr. Each subject received 400 mg ibuprofen or placebo.

Figure 27. Mean body temperature and melatonin change scores (+/- SEM), calculated as difference from baseline measurement at 2300 hr for placebo (o---o) and ibuprofen (•—•). A cross (+) denotes that body temperature in the ibuprofen group was significantly different ($p < .05$) from that in the placebo group. An asterisk (*) denotes that melatonin in the ibuprofen group was significantly different ($p < .05$) from that in the placebo group. A total of 10 subjects were tested on two occasions from 2300 to 0300 hr. Each subject received 400 or 650 mg ibuprofen on one occasion and placebo on another (in a counterbalanced order).

Figure 28. Alpha EEG: Relative spectral power as a function of Night, Condition, and Time of Night.

Figure 29. Theta EEG: Relative spectral power as a function of Night, Condition, and Time of Night.

Figure 30. Maintenance of Wakefulness Test: Stage 2 latency as a function of Night, Condition, and Time of Night.

Figure 31. Stanford Sleepiness Scale: Subjective sleepiness as a function of Night, Condition, and Time of Night.

Figure 32. Continuous Recognition Task: Throughput as a function of Night, Condition, and Time of Night.

Figure 33. Dual Task: Number of control losses as a function of Night, Condition, and Time of Night.

Figure 34. Dual Task: Throughput as a function of Night, Condition, and Time of Night.

Figure 35. Probed Memory Recall Test: Number of words recalled as a function of Night, Condition, and Time of Night.

Figure 36. Procedural Memory Task (Basic): Throughput as a function of Night, Condition, and Time of Night.

Figure 37. Procedural Memory Task (Coded): Throughput as a function of Night, Condition, and Time of Night.

Figure 38. Switching Task (Mannequin): Throughput as a function of Night, Condition, and Time of Night.

Figure 39. Switching Task (Processing): Throughput as a function of Night, Condition, and Time of Night.

Figure 40. Area under the Curve salivary melatonin levels (Log [x+1]) for dim-light placebo (DLP), dim-light caffeine (DLC), bright-light placebo (BLP) and bright-light caffeine (BLC) on both nights of sleep deprivation. Melatonin levels are lower under caffeine and bright light treatments compared to dim-light placebo. The combined treatment of bright light and caffeine resulted in the lowest melatonin levels both nights.

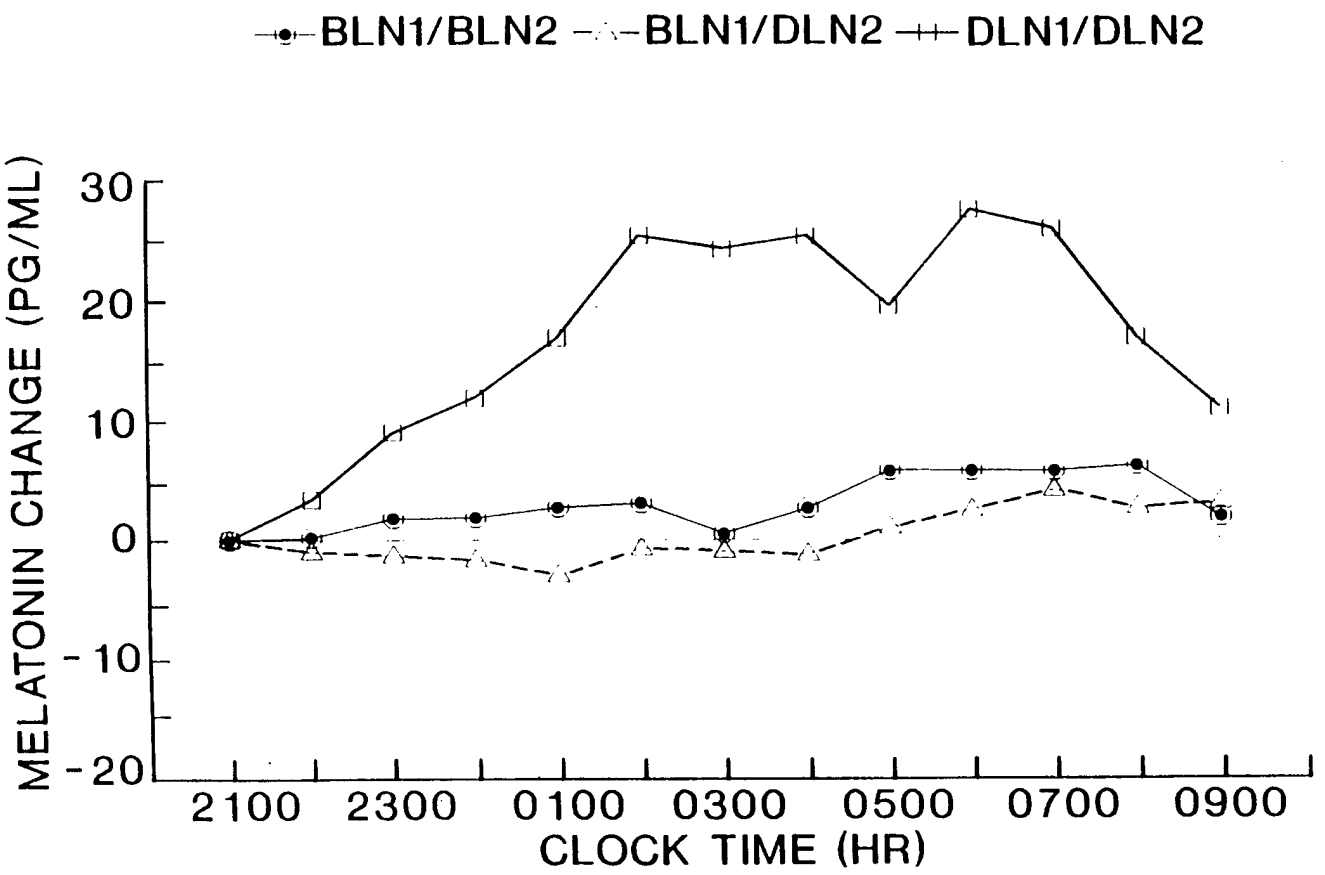
Figure 41. Average tympanic temperature change (difference from 2000 hr baseline) for dim-light placebo (DLP), dim-light caffeine (DLC), bright-light placebo (BLP) and bright-light caffeine (BLC) on both nights of sleep deprivation. Tympanic temperature is higher under caffeine and bright light treatments compared to dim-light placebo. The combined treatment of bright light and caffeine resulted in the highest temperature levels both nights.

Figure 42. Spectral EEG data for Delta and Theta absolute power. Little systematic effect of the treatments is observed.

Figure 43. Spectral EEG data for Alpha and Beta absolute power. Little systematic effect of the treatments is observed.

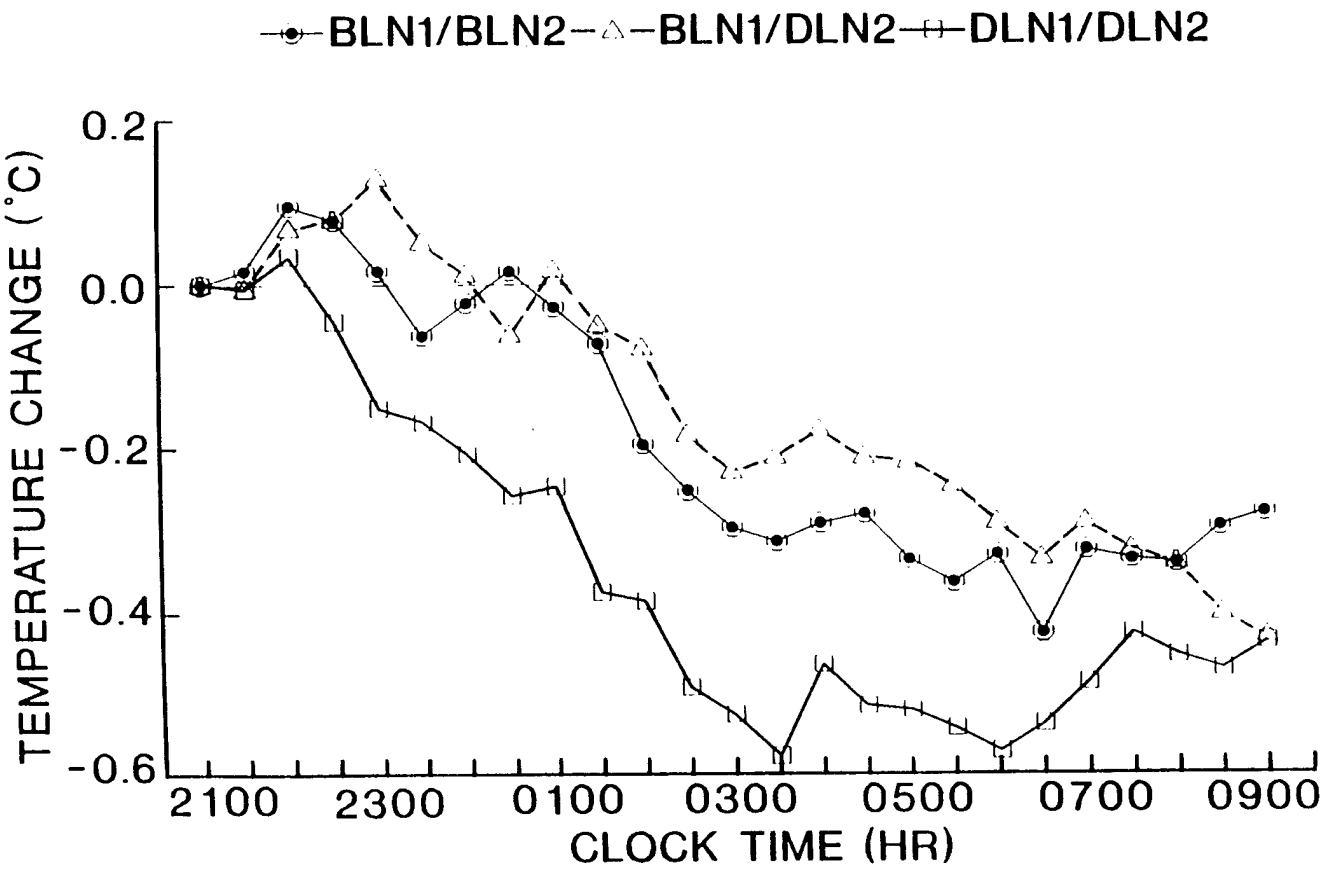
Figure 1

NIGHT 1: MELATONIN



BLN1/DLN2 DIFFERS FROM DLN1/DLN2; P < .05
BLN1/BLN2 DIFFERS FROM DLN1/DLN2; P < .05
BLN1/DLN2 DOES NOT DIFFER FROM BLN1/BLN2; P > .05

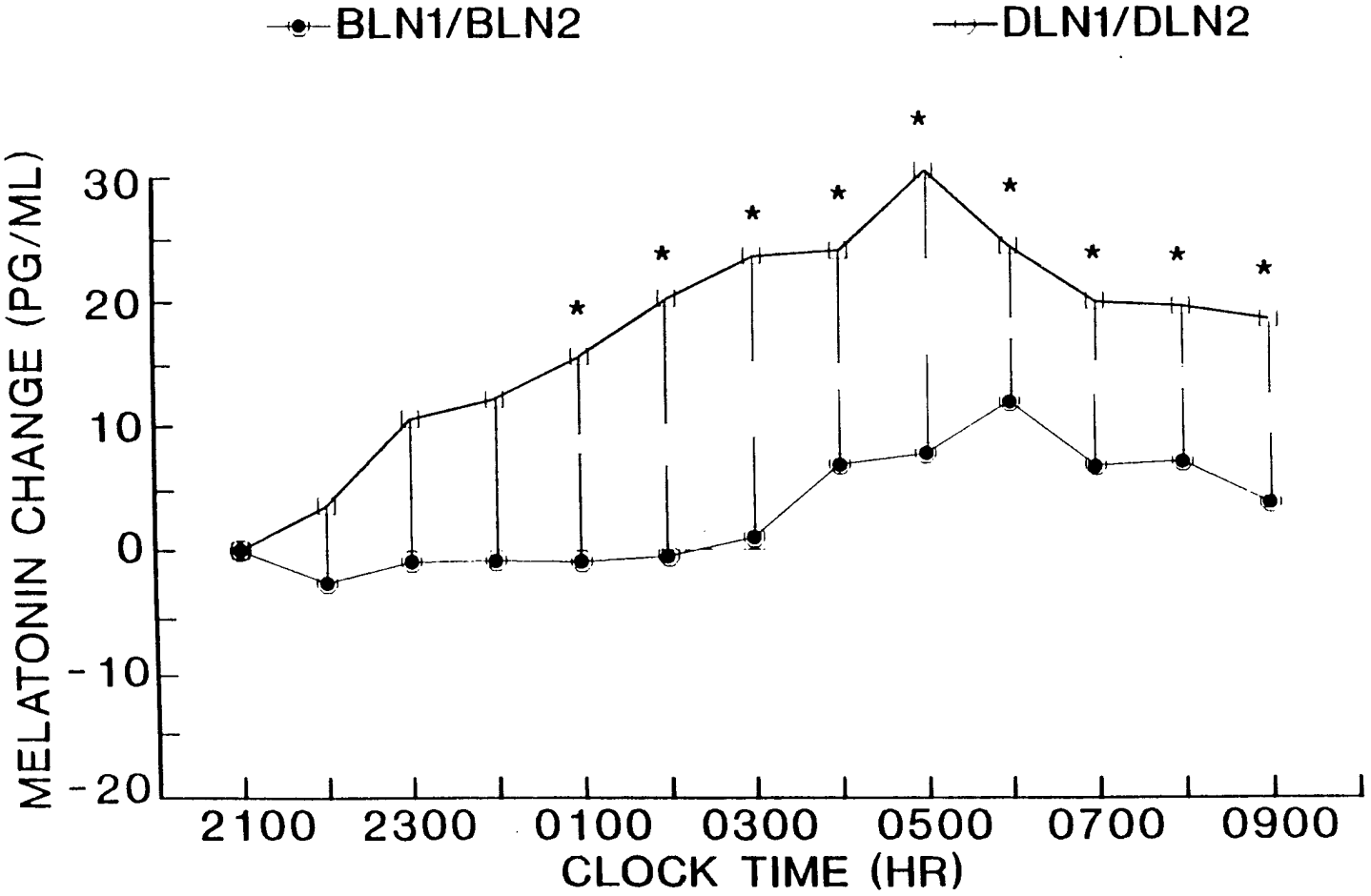
Figure 2
NIGHT 1: TEMPERATURE



BLN1/DLN2 DIFFERS FROM DLN1/DLN2; P < .05
BLN1/BLN2 DIFFERS FROM DLN1/DLN2; P < .05
BLN1/DLN2 DOES NOT DIFFER FROM BLN1/BLN2; P > .05

Figure 3

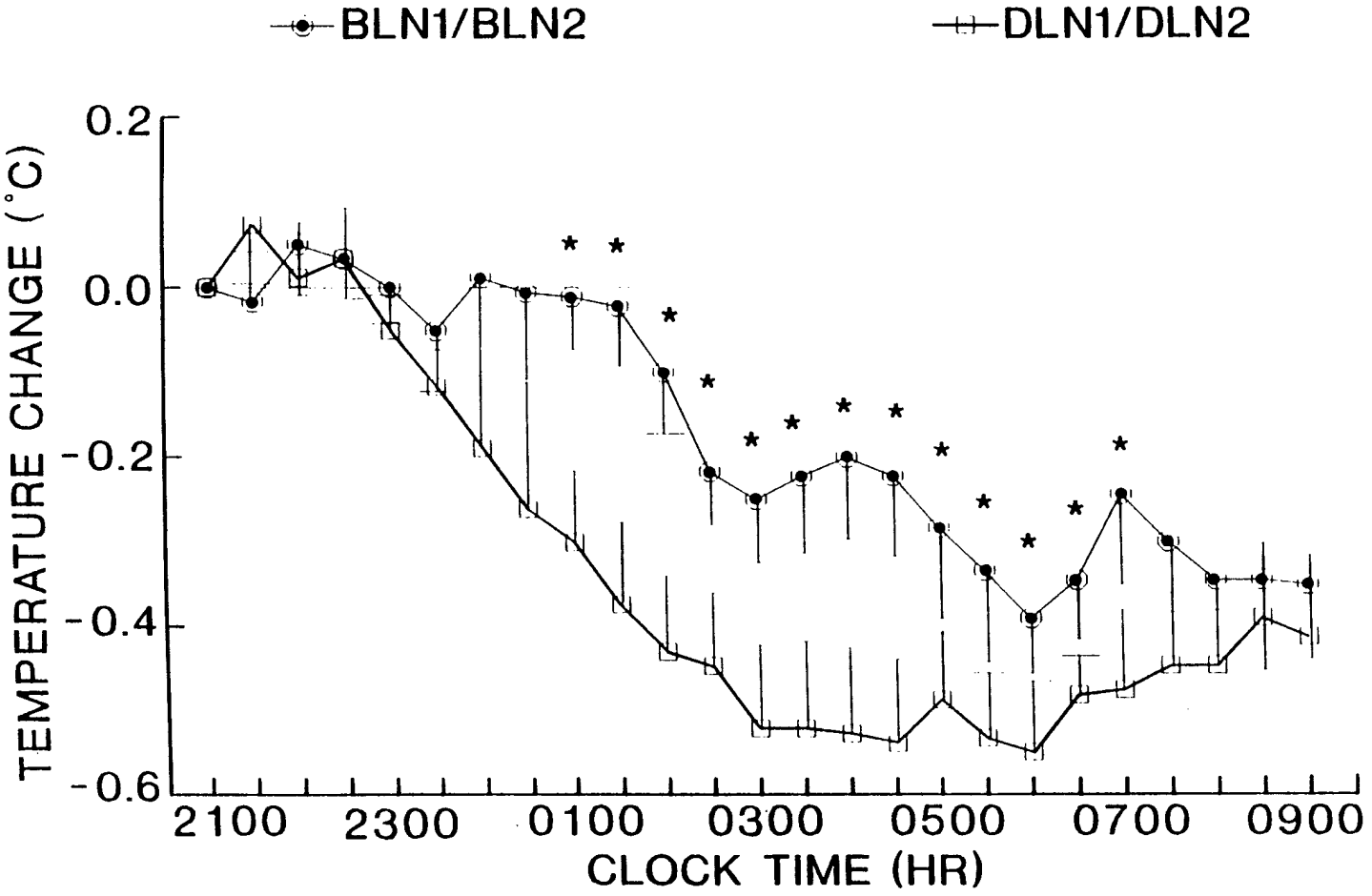
NIGHT 2: MELATONIN



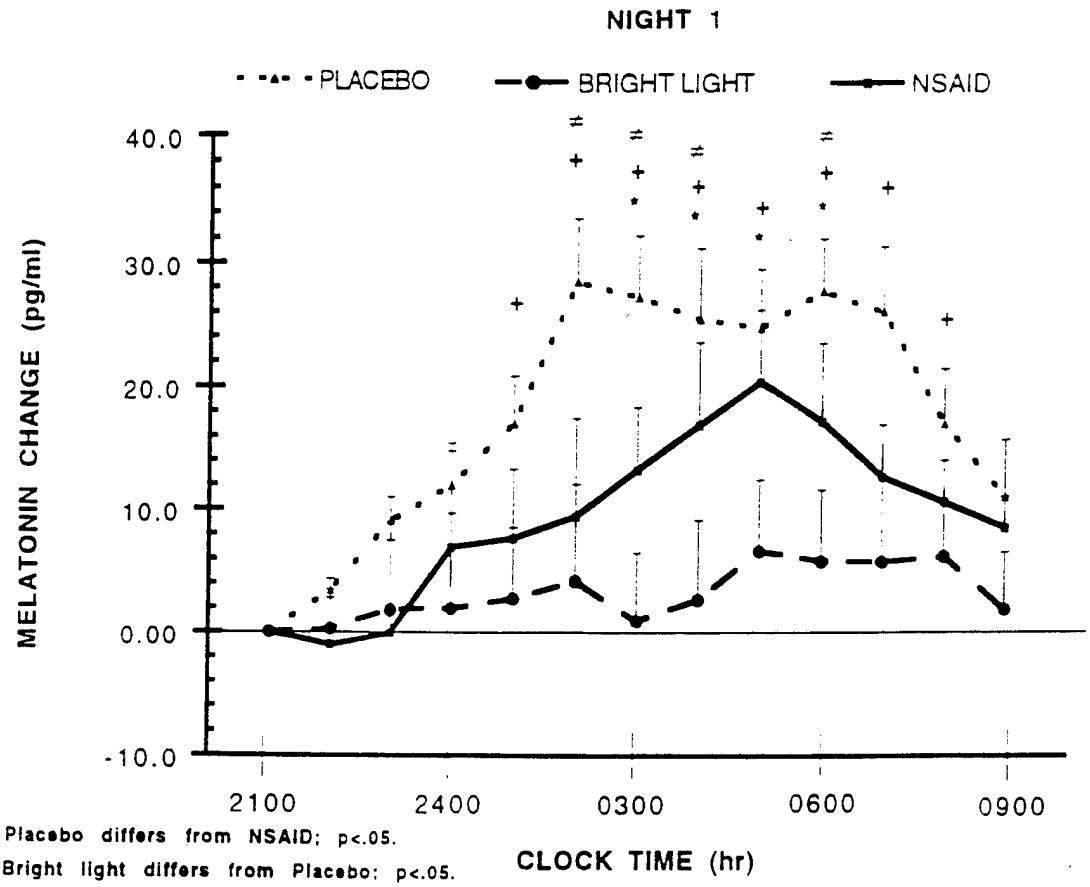
* BRIGHT LIGHT DIFFERS FROM DIM LIGHT; P < .05

Figure 4

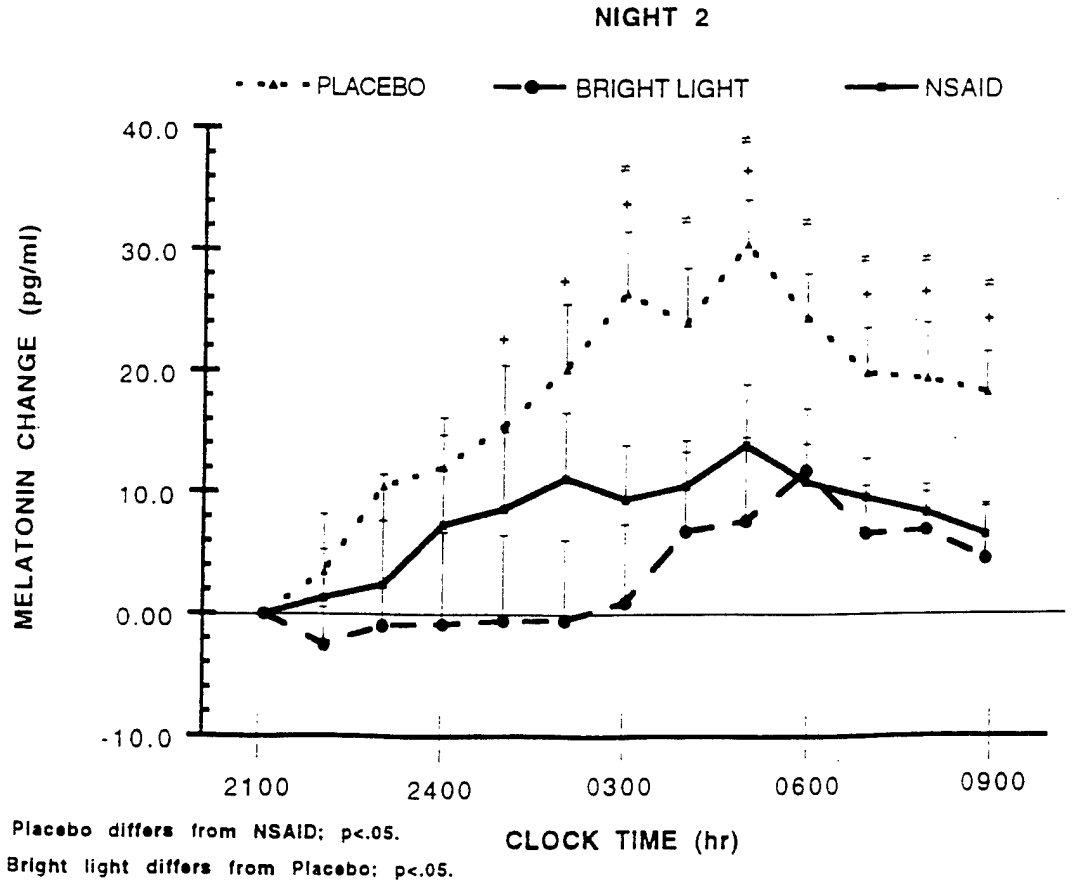
NIGHT 2: TEMPERATURE



* BRIGHT LIGHT DIFFERS FROM DIM LIGHT; P < .05

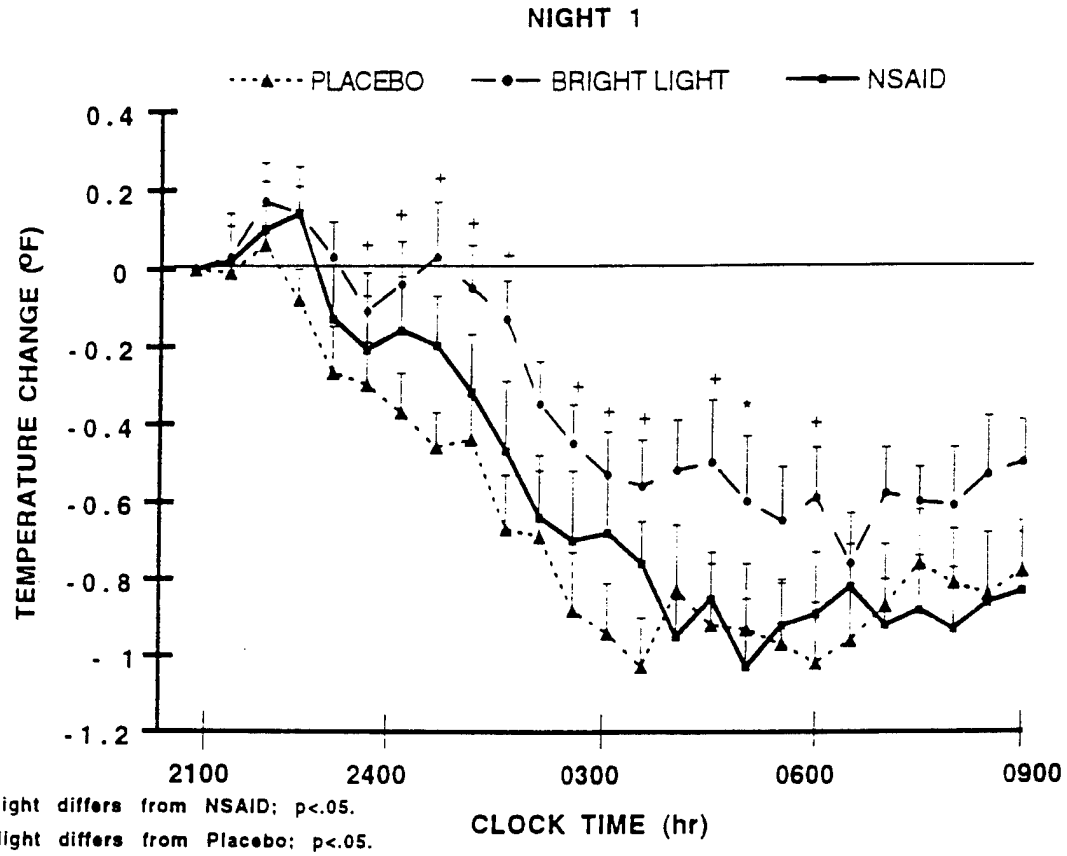


· Placebo differs from NSAID; p<.05.
· Bright light differs from Placebo; p<.05.
· Bright light differs from NSAID; p<.05.

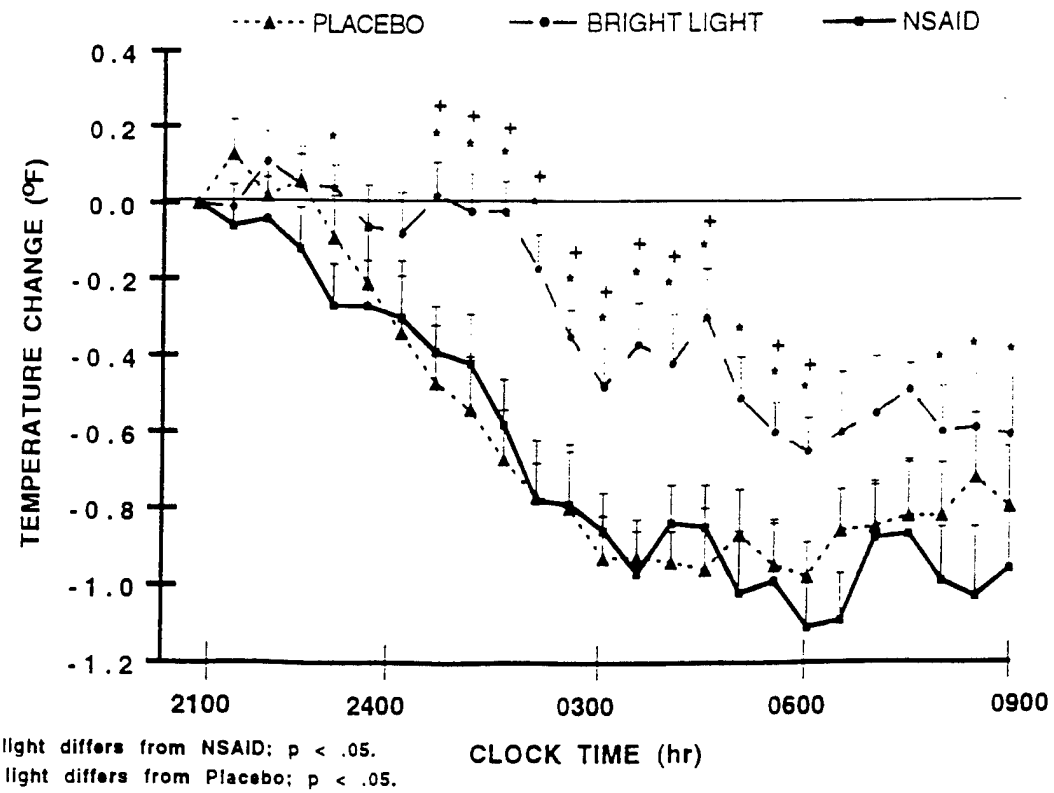


· Placebo differs from NSAID; p<.05.
· Bright light differs from Placebo; p<.05.

Figure 5



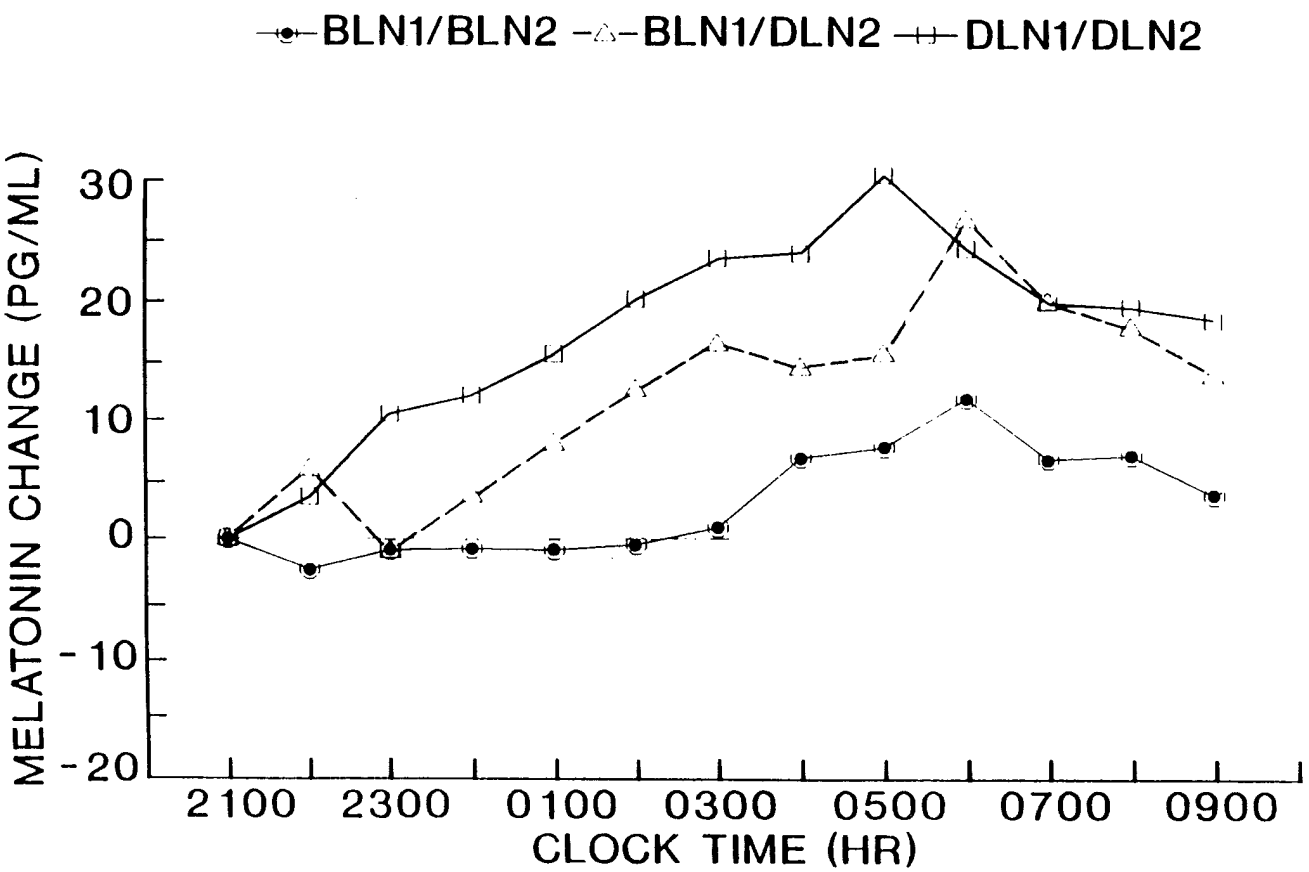
Bright light differs from NSAID: p<.05.
Bright light differs from Placebo: p<.05.



Bright light differs from NSAID: p < .05.
Bright light differs from Placebo: p < .05.

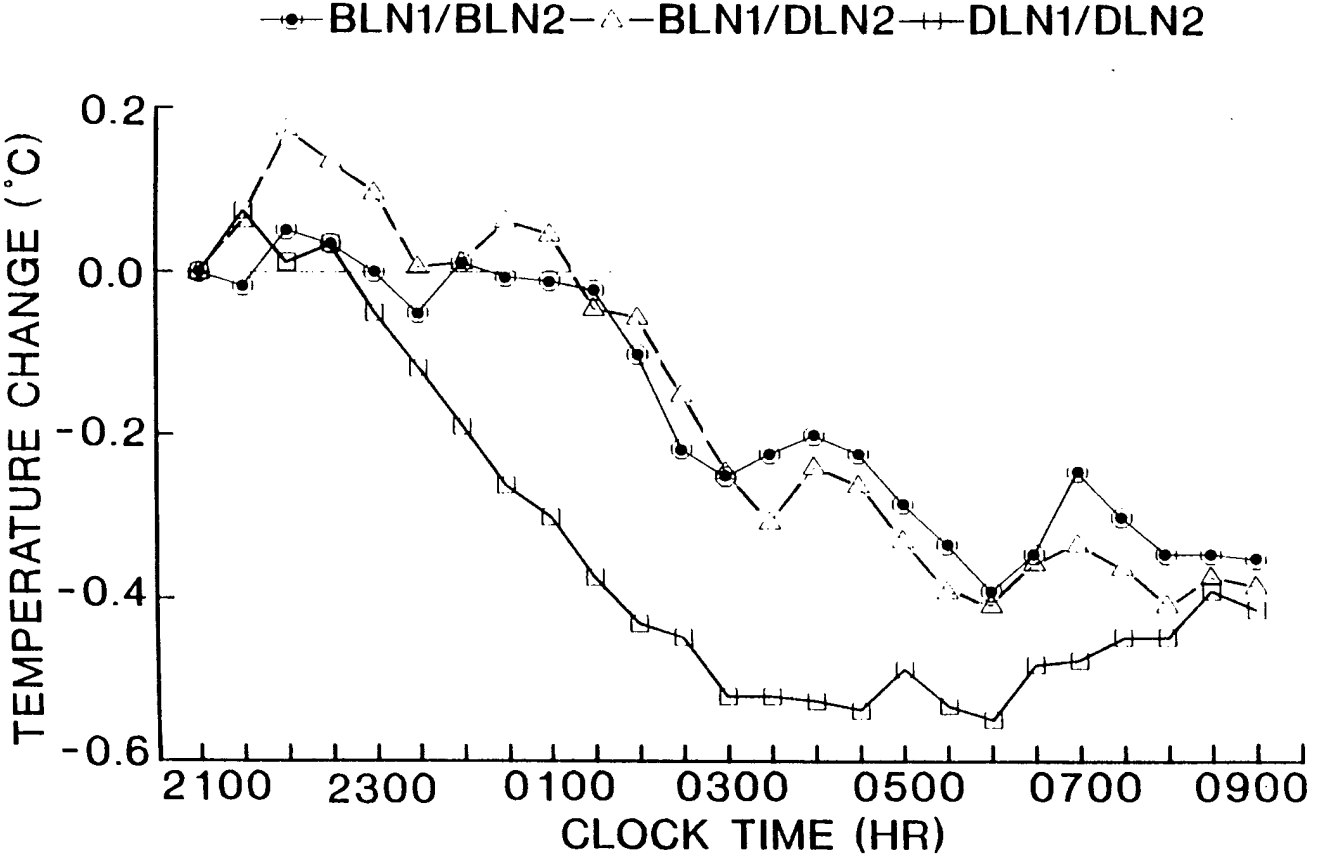
Figure 6

Figure 7
NIGHT 2: MELATONIN



BLN1/DLN2 DOES NOT DIFFER FROM DLN1/DLN2; P > .05
BLN1/BLN2 DIFFERS FROM DLN1/DLN2; P < .05
BLN1/DLN2 DIFFERS FROM BLN1/BLN2; P < .05

Figure 8
NIGHT 2: TEMPERATURE



BLN1/DLN2 DIFFERS FROM DLN1/DLN2; P < .05
BLN1/BLN2 DIFFERS FROM DLN1/DLN2; P < .05
BLN1/DLN2 DOES NOT DIFFER FROM BLN1/BLN2; P > .05

Figure 9

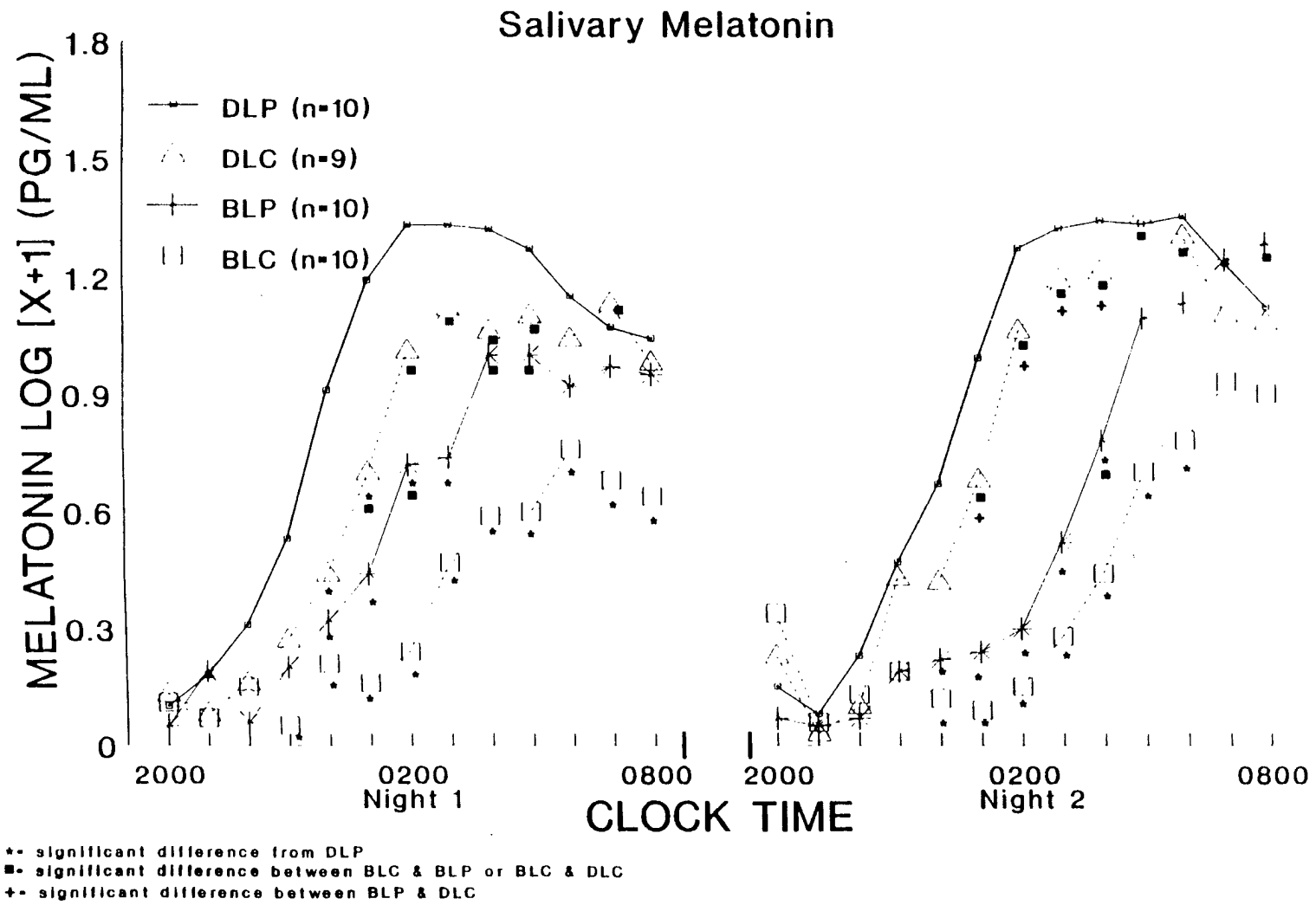

Salivary Melatonin
MELATONIN LOG [X+1] (PG/ML)
1.8
1.5
1.2
0.9
0.6
0.3
0
DLP (n=10)
DLC (n=9)
BLP (n=10)
BLC (n=10)
2000
0200
0800
Night 1
2000
0200
0800
Night 2
CLOCK TIME
*- significant difference from DLP
■- significant difference between BLC & BLP or BLC & DLC
+- significant difference between BLP & DLC

Figure 10

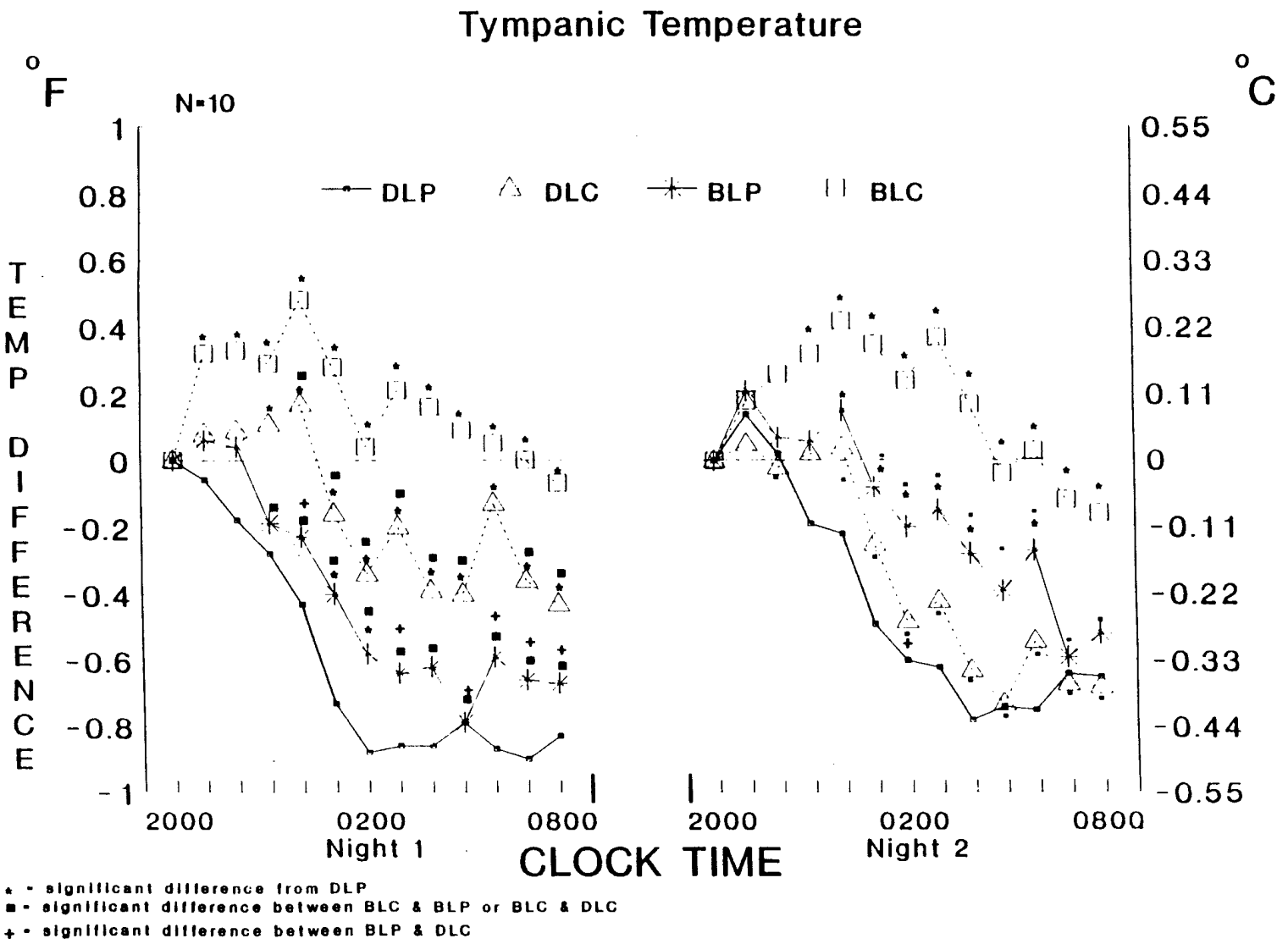

Tympanic Temperature
°F
°C
N=10
DLP
DLC
BLP
BLC
TEMP DIFFERENCE
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
0.55
0.44
0.33
0.22
0.11
0
-0.11
-0.22
-0.33
-0.44
-0.55
2000
0200
0800
Night 1
2000
0200
0800
Night 2
CLOCK TIME
* - significant difference from DLP
■ - significant difference between BLC & BLP or BLC & DLC
+ - significant difference between BLP & DLC

Figure 11

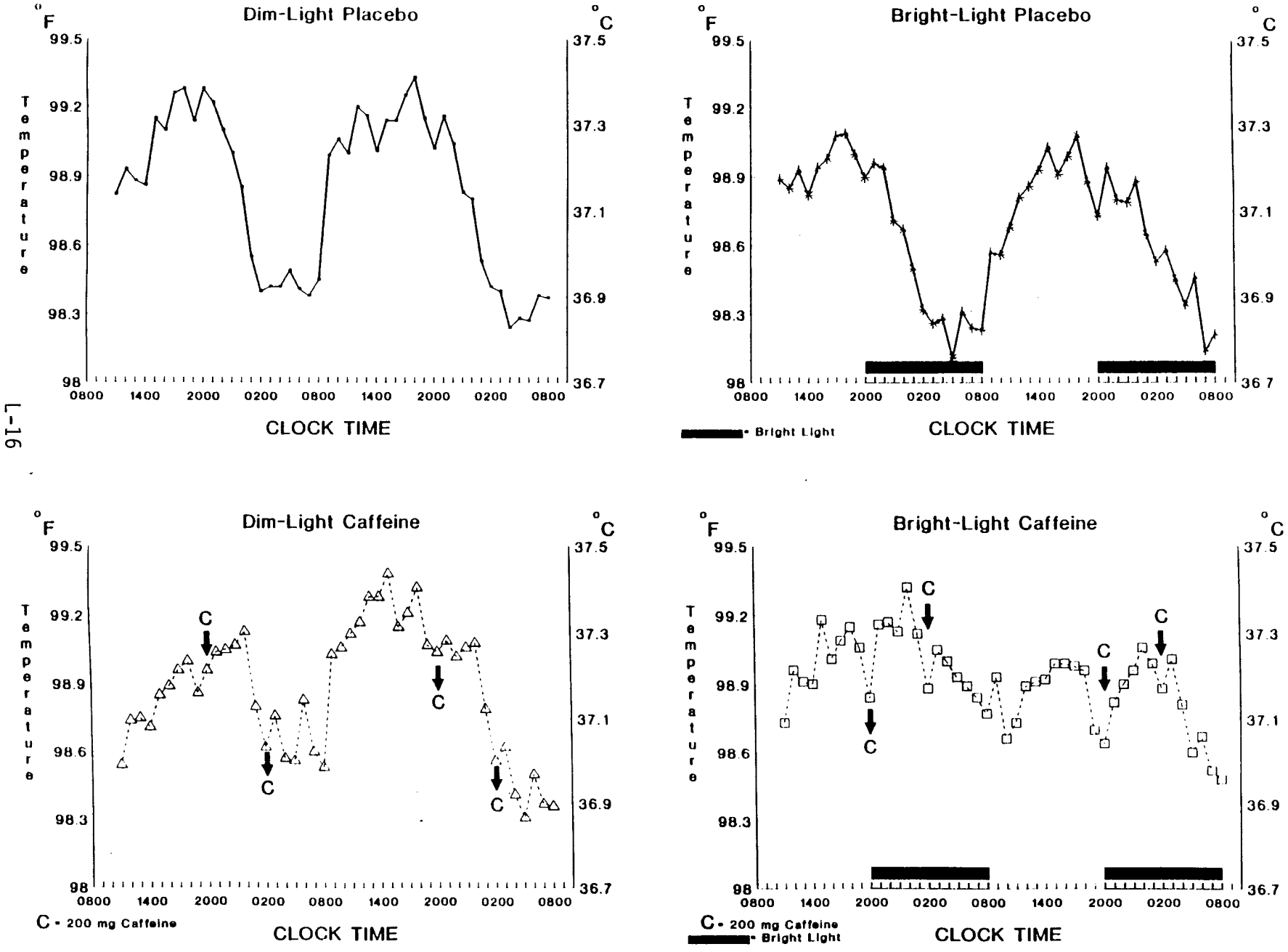

Dim-Light Placebo
°F
°C
99.5
99.2
98.9
98.6
98.3
98
37.5
37.3
37.1
36.9
36.7
Temperature
0800 1400 2000 0200 0800 1400 2000 0200 0800
CLOCK TIME
Bright-Light Placebo
Bright Light
Dim-Light Caffeine
C
C - 200 mg Caffeine
Bright-Light Caffeine

Figure 12

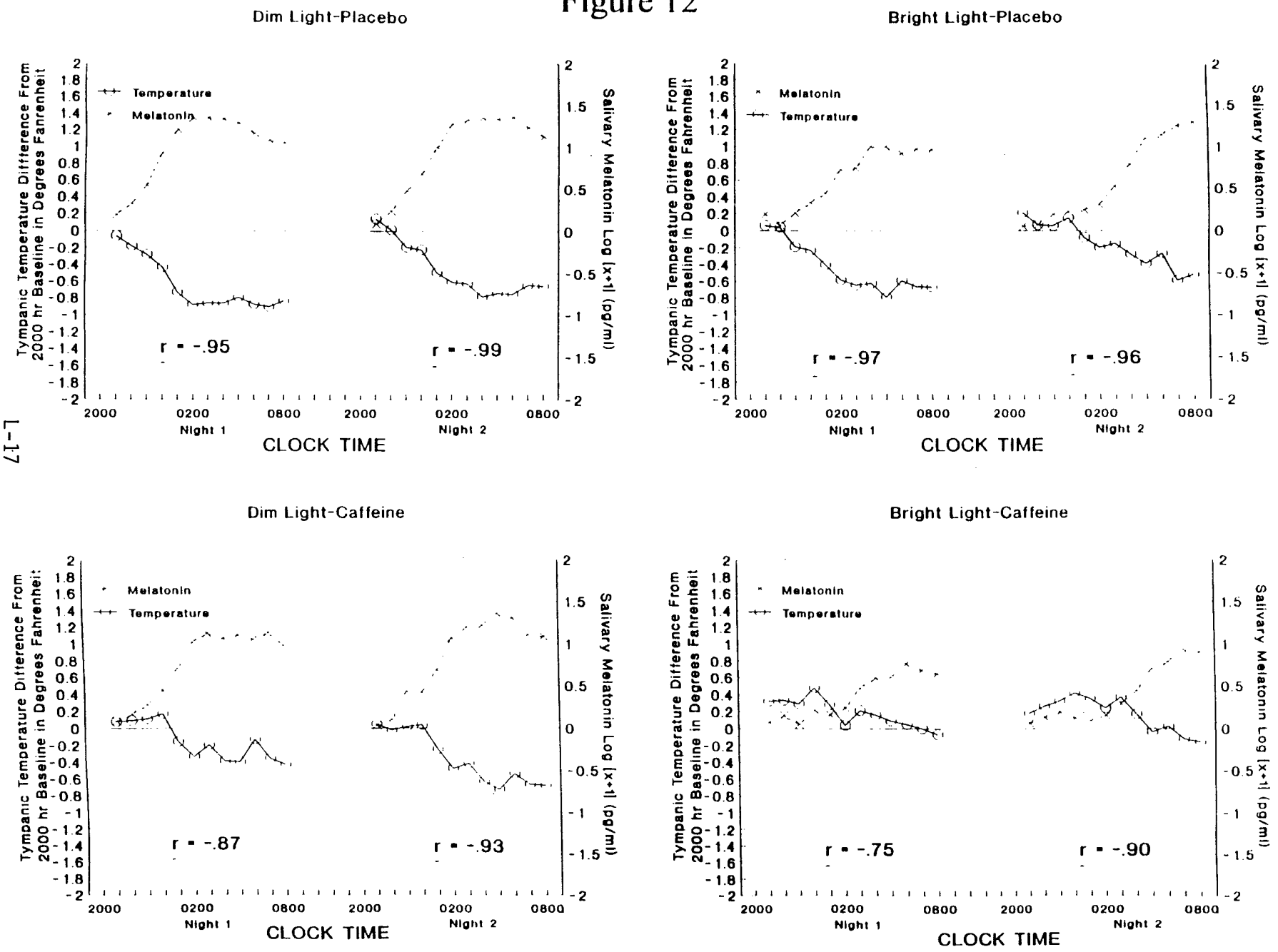

Dim Light-Placebo
Bright Light-Placebo
Dim Light-Caffeine
Bright Light-Caffeine
Temperature
Melatonin
Tympanic Temperature Difference From 2000 hr Baseline in Degrees Fahrenheit
Salivary Melatonin Log [x+1] (pg/ml)
r = -.95
r = -.99
r = -.97
r = -.96
r = -.87
r = -.93
r = -.75
r = -.90
2000
0200
0800
Night 1
Night 2
CLOCK TIME

Figure 13

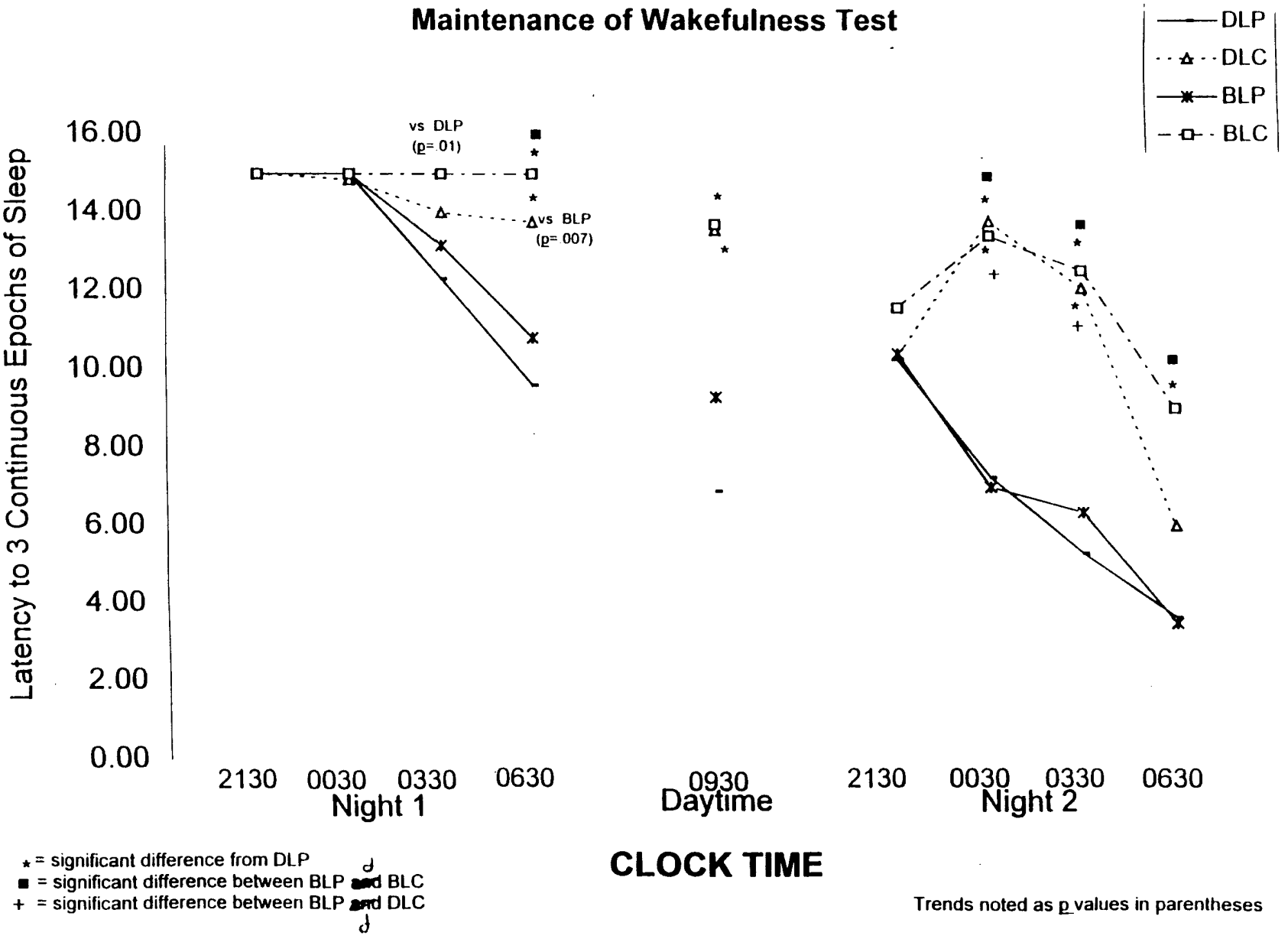

Maintenance of Wakefulness Test
DLP
DLC
BLP
BLC
Latency to 3 Continuous Epochs of Sleep
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
vs DLP
(p=.01)
vs BLP
(p=.007)
2130
0030
0330
0630
Night 1
0930
Daytime
2130
0030
0330
0630
Night 2
CLOCK TIME
* = significant difference from DLP
■ = significant difference between BLP and BLC
+ = significant difference between BLP and DLC
Trends noted as p values in parentheses

Figure 14

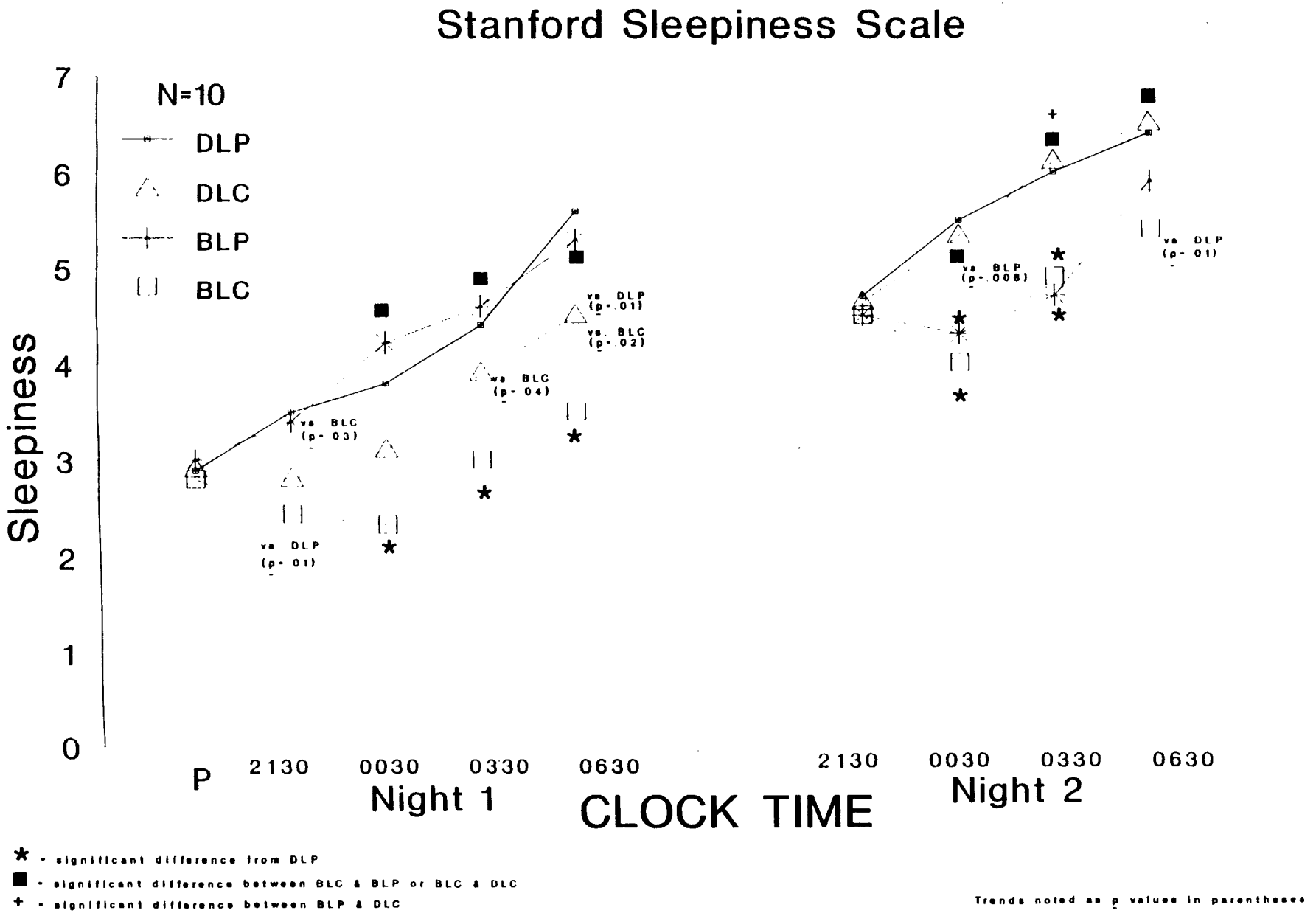

Stanford Sleepiness Scale
N=10
DLP
DLC
BLP
BLC
Sleepiness
7
6
5
4
3
2
1
0
vs DLP (p-.01)
vs BLC (p-.03)
vs BLC (p-.04)
vs DLP (p-.01)
vs BLC (p-.02)
vs BLP (p-.006)
vs DLP (p-.01)
P
2130
0030
0330
0630
Night 1
CLOCK TIME
2130
0030
0330
0630
Night 2
* - significant difference from DLP
■ - significant difference between BLC & BLP or BLC & DLC
+ - significant difference between BLP & DLC
Trends noted as p values in parentheses

Figure 15

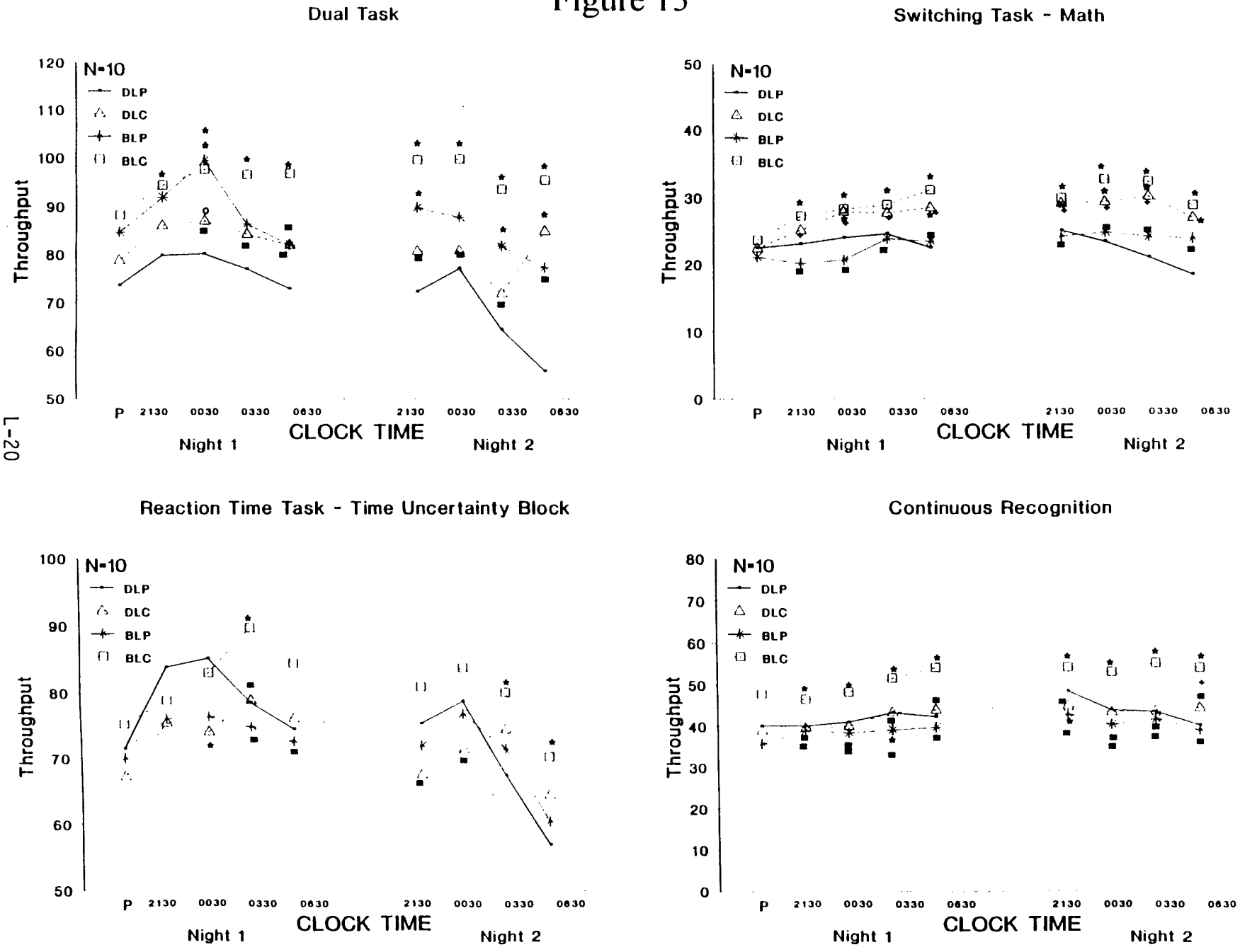

Dual Task
N=10
DLP
DLC
BLP
BLC
Throughput
120
110
100
90
80
70
60
50
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2
Switching Task - Math
N=10
DLP
DLC
BLP
BLC
Throughput
50
40
30
20
10
0
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2
Reaction Time Task - Time Uncertainty Block
N=10
DLP
DLC
BLP
BLC
Throughput
100
90
80
70
60
50
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2
Continuous Recognition
N=10
DLP
DLC
BLP
BLC
Throughput
80
70
60
50
40
30
20
10
0
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2

Figure 16

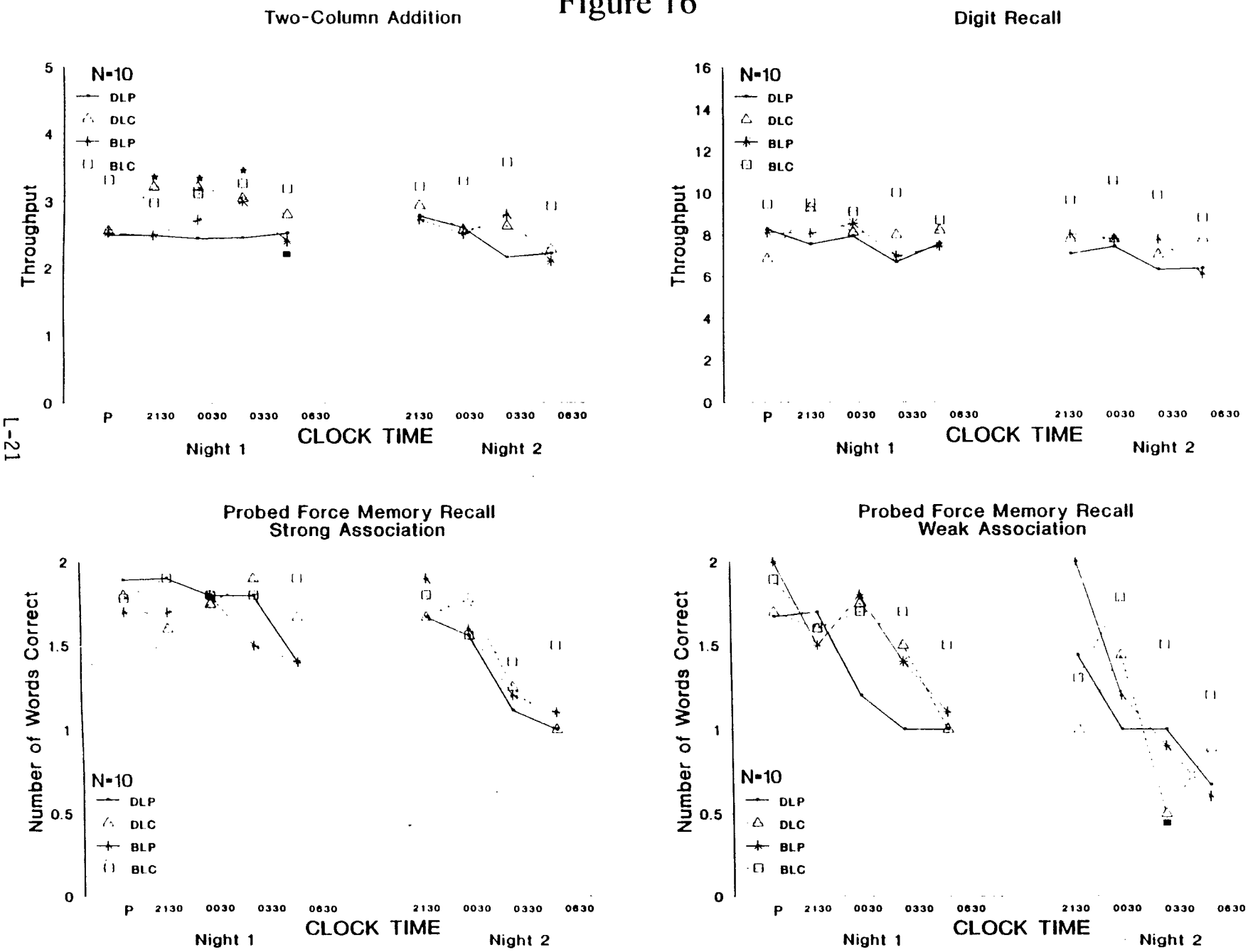

Two-Column Addition
N=10
DLP
DLC
BLP
BLC
Throughput
0
1
2
3
4
5
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2
Digit Recall
N=10
DLP
DLC
BLP
BLC
Throughput
0
2
4
6
8
10
12
14
16
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2
Probed Force Memory Recall
Strong Association
Number of Words Correct
0
0.5
1
1.5
2
N=10
DLP
DLC
BLP
BLC
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2
Probed Force Memory Recall
Weak Association
Number of Words Correct
0
0.5
1
1.5
2
N=10
DLP
DLC
BLP
BLC
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2

Figure 17

Thurstone Task

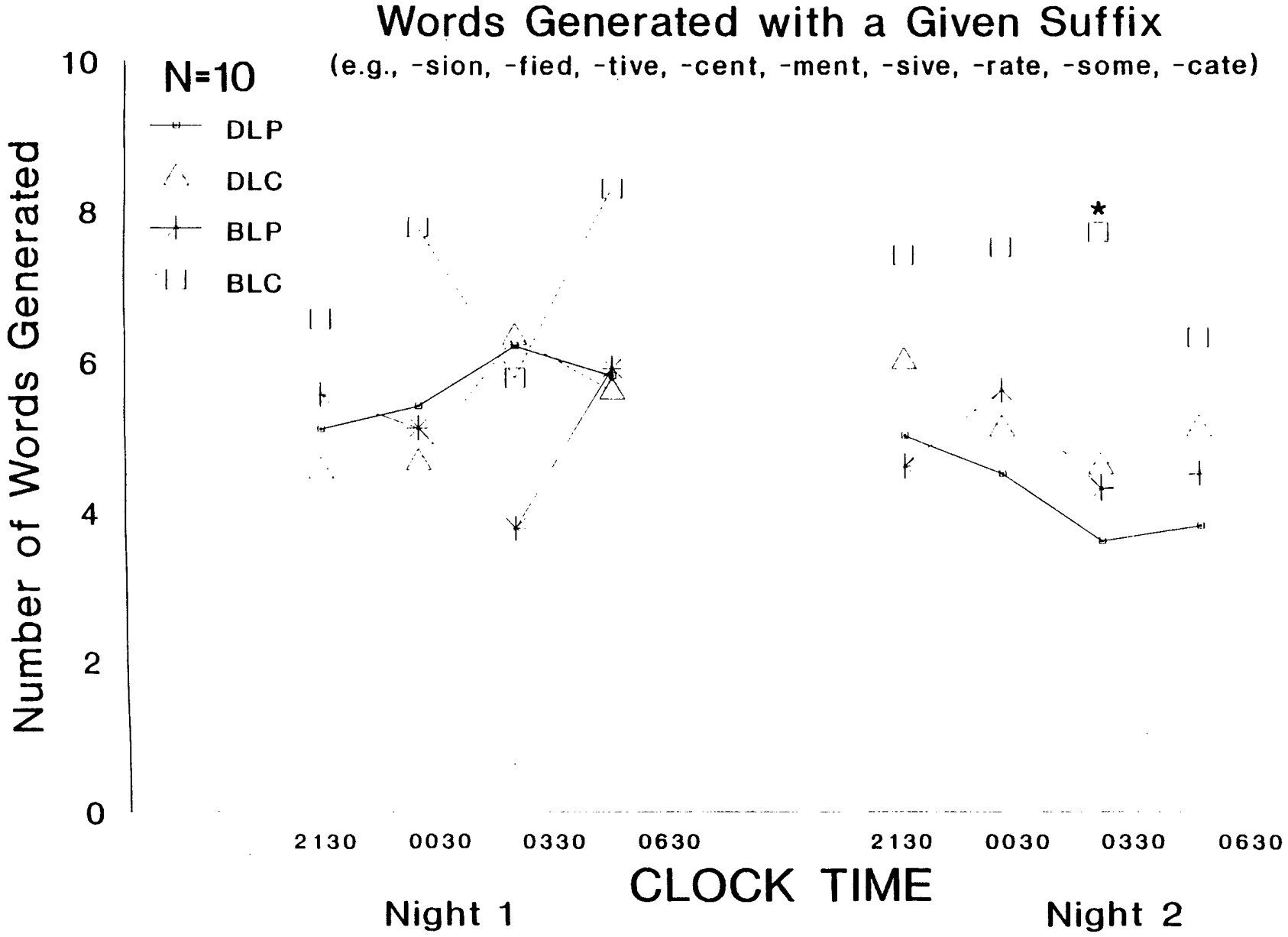
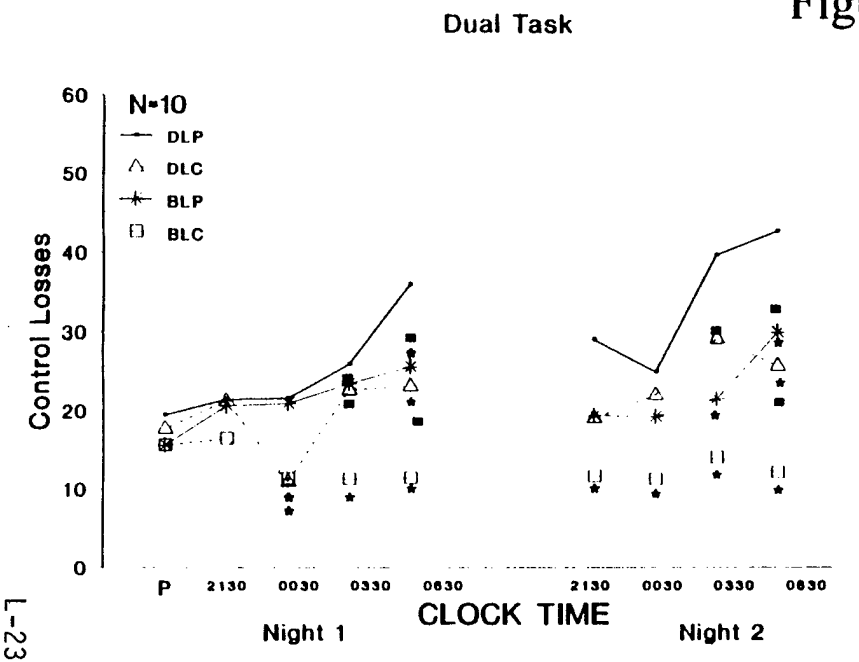
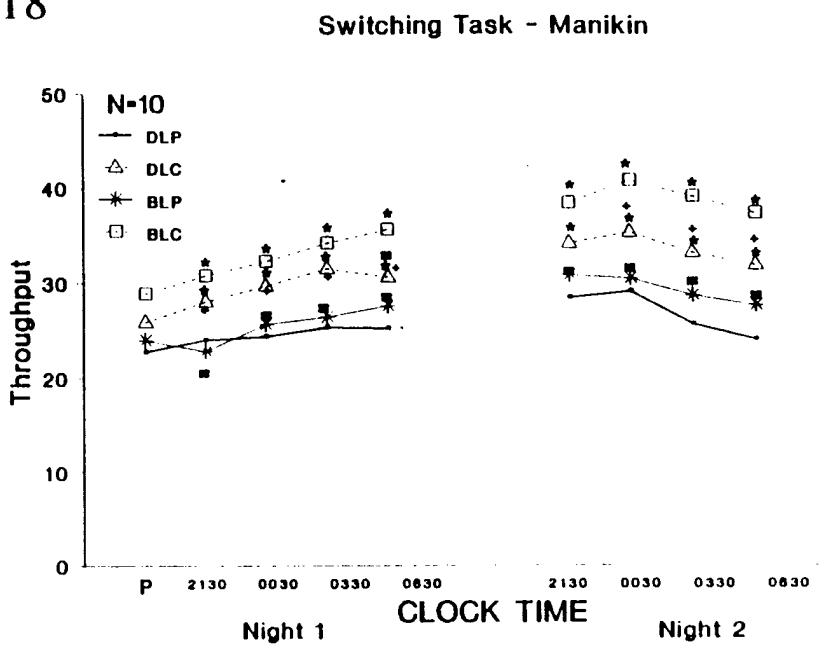
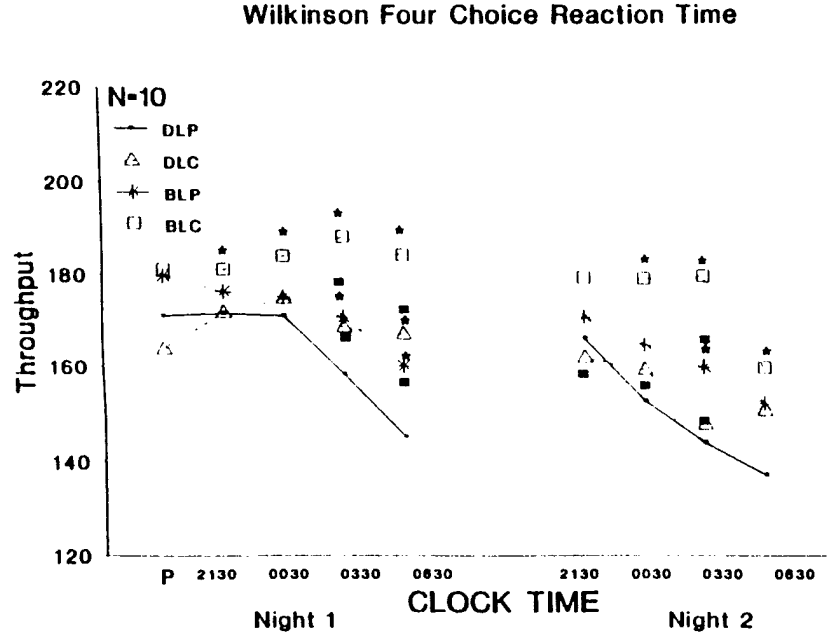


Figure 18


Dual Task
N=10
DLP
DLC
BLP
BLC
Control Losses
60
50
40
30
20
10
0
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2


Switching Task - Manikin
N=10
DLP
DLC
BLP
BLC
Throughput
50
40
30
20
10
0
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2


Wilkinson Four Choice Reaction Time
N=10
DLP
DLC
BLP
BLC
Throughput
220
200
180
160
140
120
P
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2

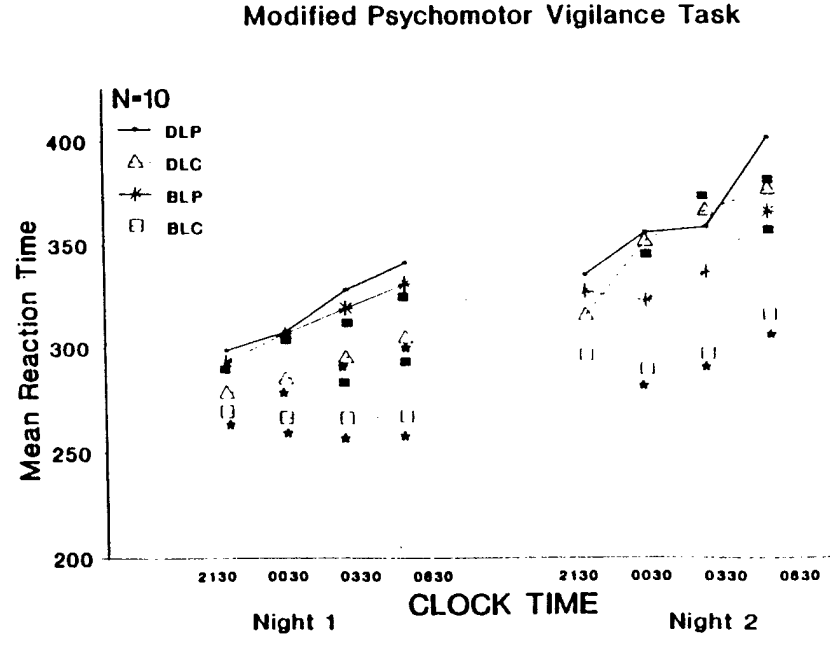

Modified Psychomotor Vigilance Task
N=10
DLP
DLC
BLP
BLC
Mean Reaction Time
400
350
300
250
200
2130
0030
0330
0630
2130
0030
0330
0630
Night 1
CLOCK TIME
Night 2

Figure 19

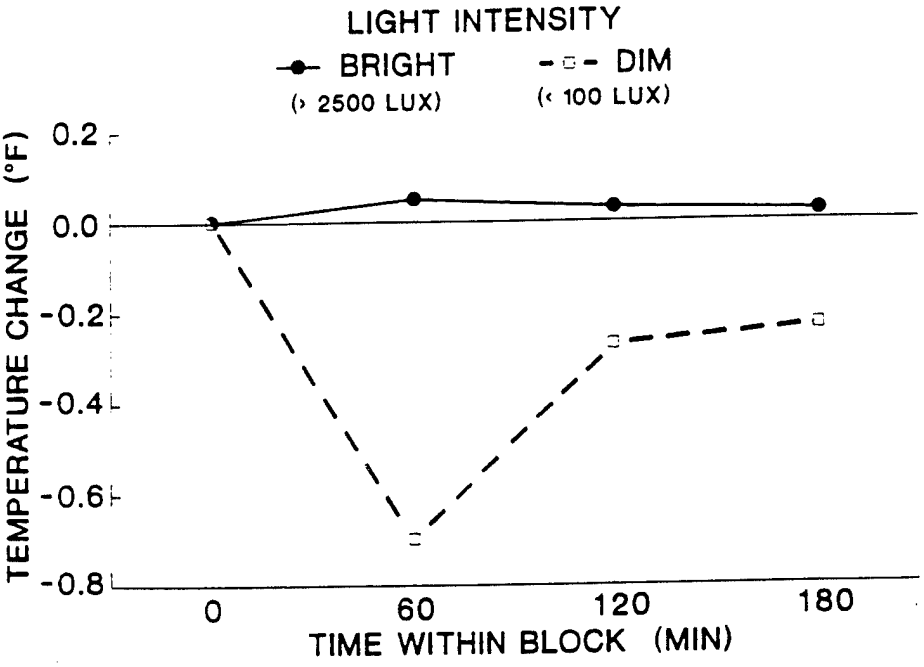


Figure 20

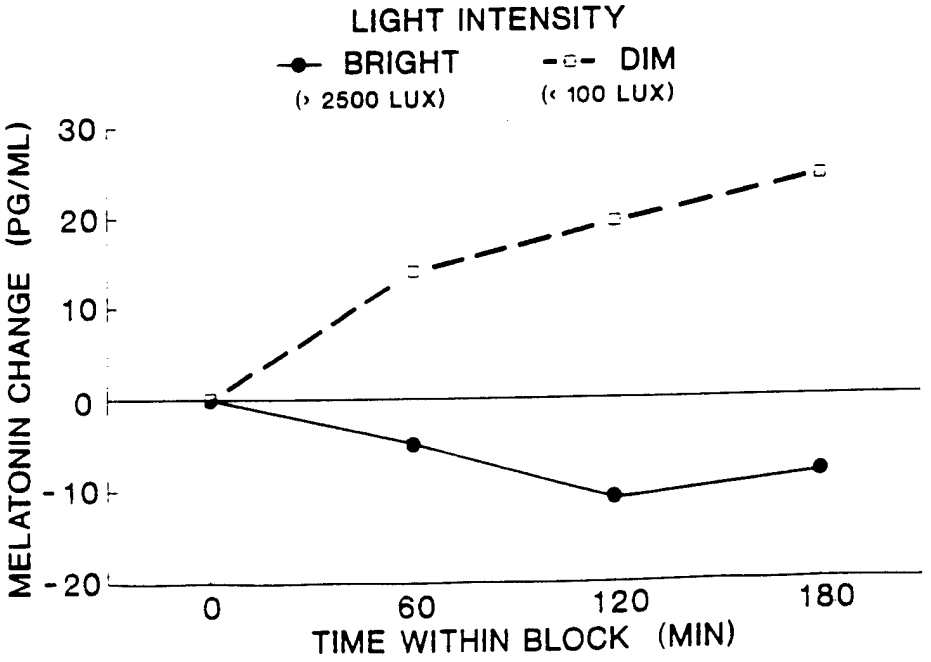
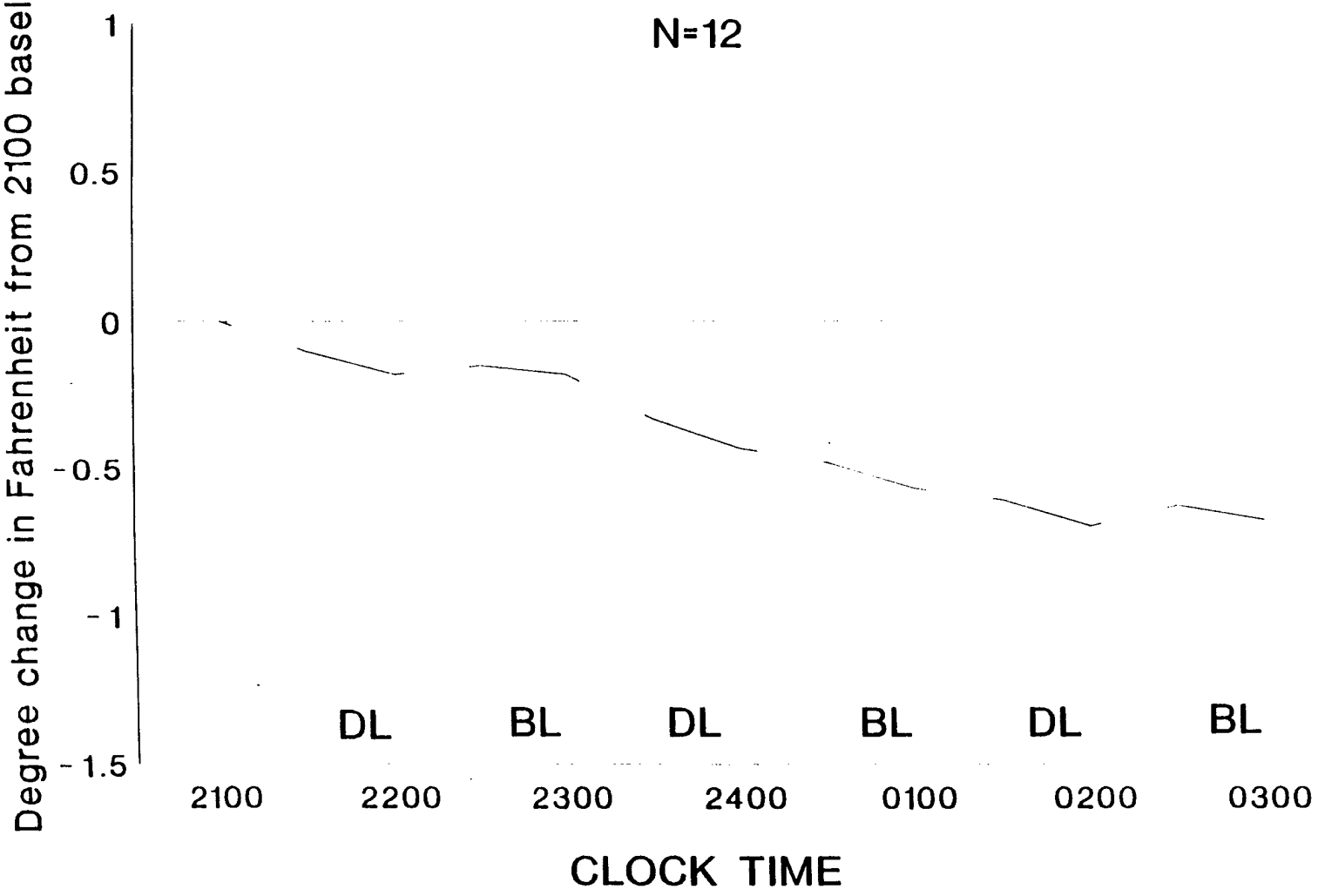
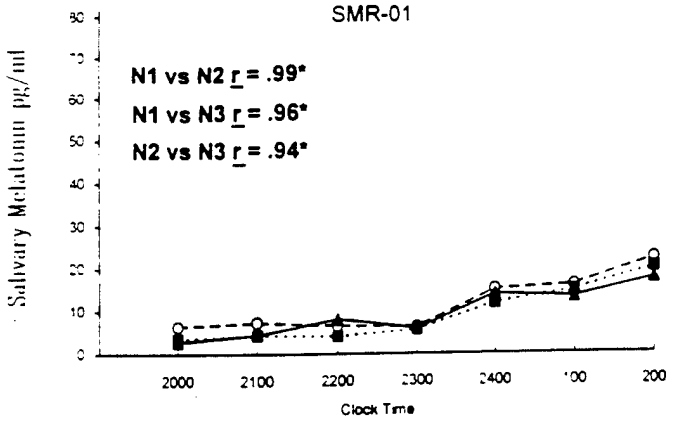


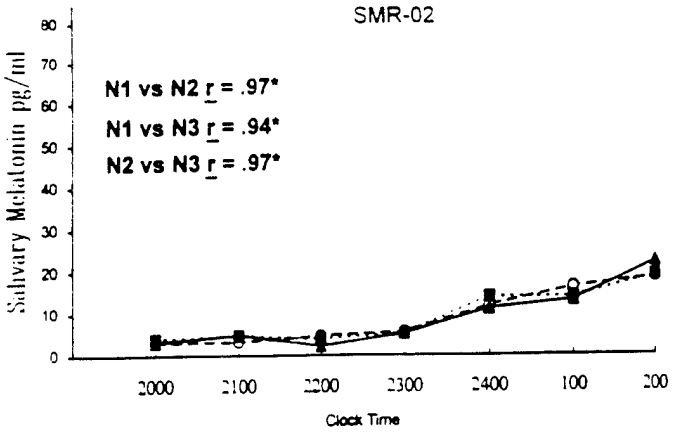
Figure 21

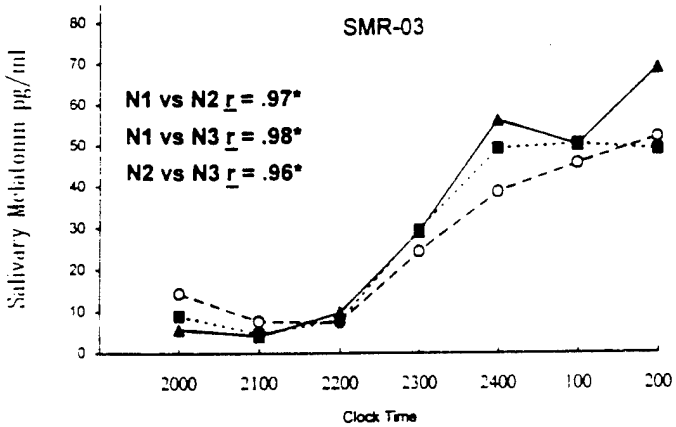
Tympanic Temperature

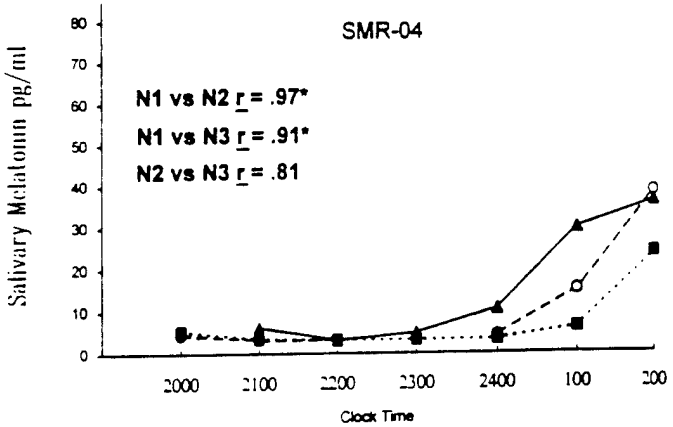
N=12

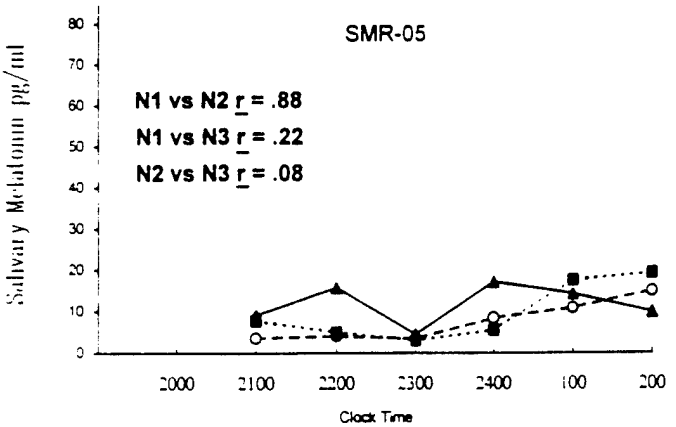


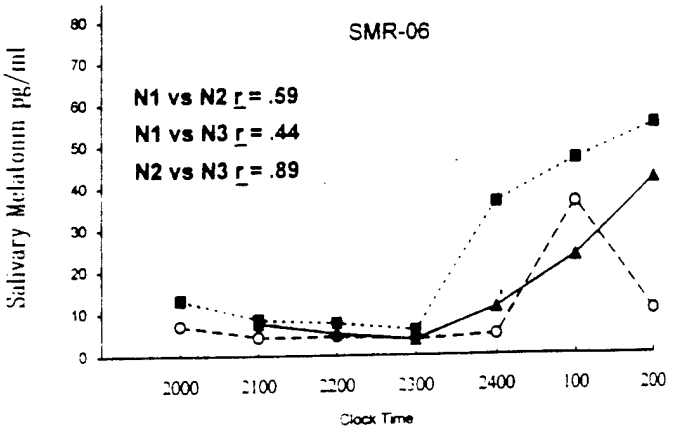

SMR-01
N1 vs N2 r = .99*
N1 vs N3 r = .96*
N2 vs N3 r = .94*
Salivary Melatonin pg/ml
Clock Time


SMR-02
N1 vs N2 r = .97*
N1 vs N3 r = .94*
N2 vs N3 r = .97*
Salivary Melatonin pg/ml
Clock Time


SMR-03
N1 vs N2 r = .97*
N1 vs N3 r = .98*
N2 vs N3 r = .96*
Salivary Melatonin pg/ml
Clock Time


SMR-04
N1 vs N2 r = .97*
N1 vs N3 r = .91*
N2 vs N3 r = .81
Salivary Melatonin pg/ml
Clock Time


SMR-05
N1 vs N2 r = .88
N1 vs N3 r = .22
N2 vs N3 r = .08
Salivary Melatonin pg/ml
Clock Time


SMR-06
N1 vs N2 r = .59
N1 vs N3 r = .44
N2 vs N3 r = .89
Salivary Melatonin pg/ml
Clock Time

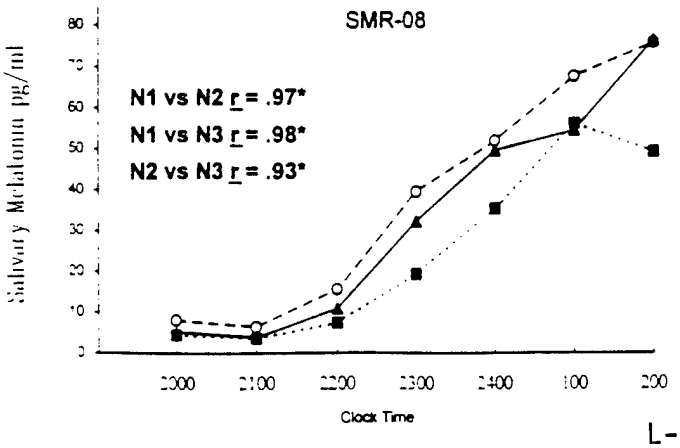

SMR-08
N1 vs N2 r = .97*
N1 vs N3 r = .98*
N2 vs N3 r = .93*
Salivary Melatonin pg/ml
Clock Time

Figure 22

Figure 23

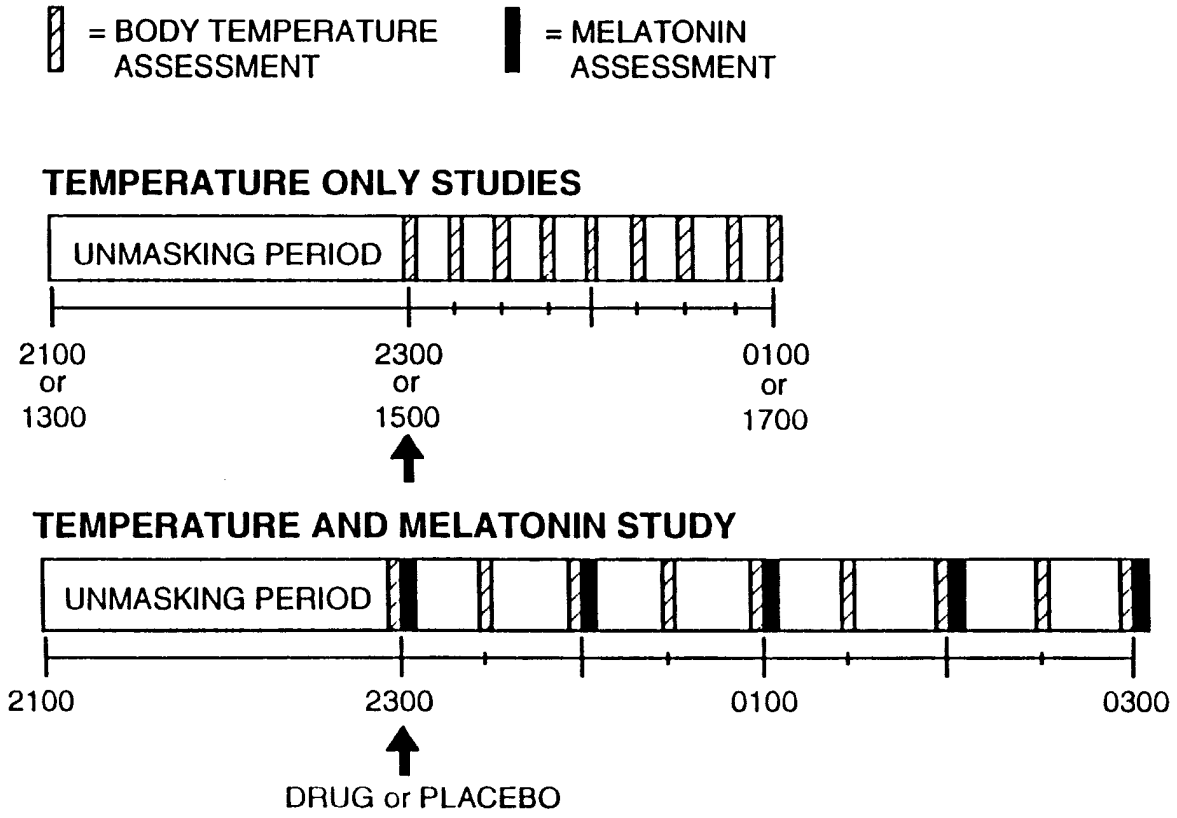

= BODY TEMPERATURE ASSESSMENT
= MELATONIN ASSESSMENT
TEMPERATURE ONLY STUDIES
UNMASKING PERIOD
2100 or 1300
2300 or 1500
0100 or 1700
TEMPERATURE AND MELATONIN STUDY
UNMASKING PERIOD
2100
2300
0100
0300
DRUG or PLACEBO

Figure 24

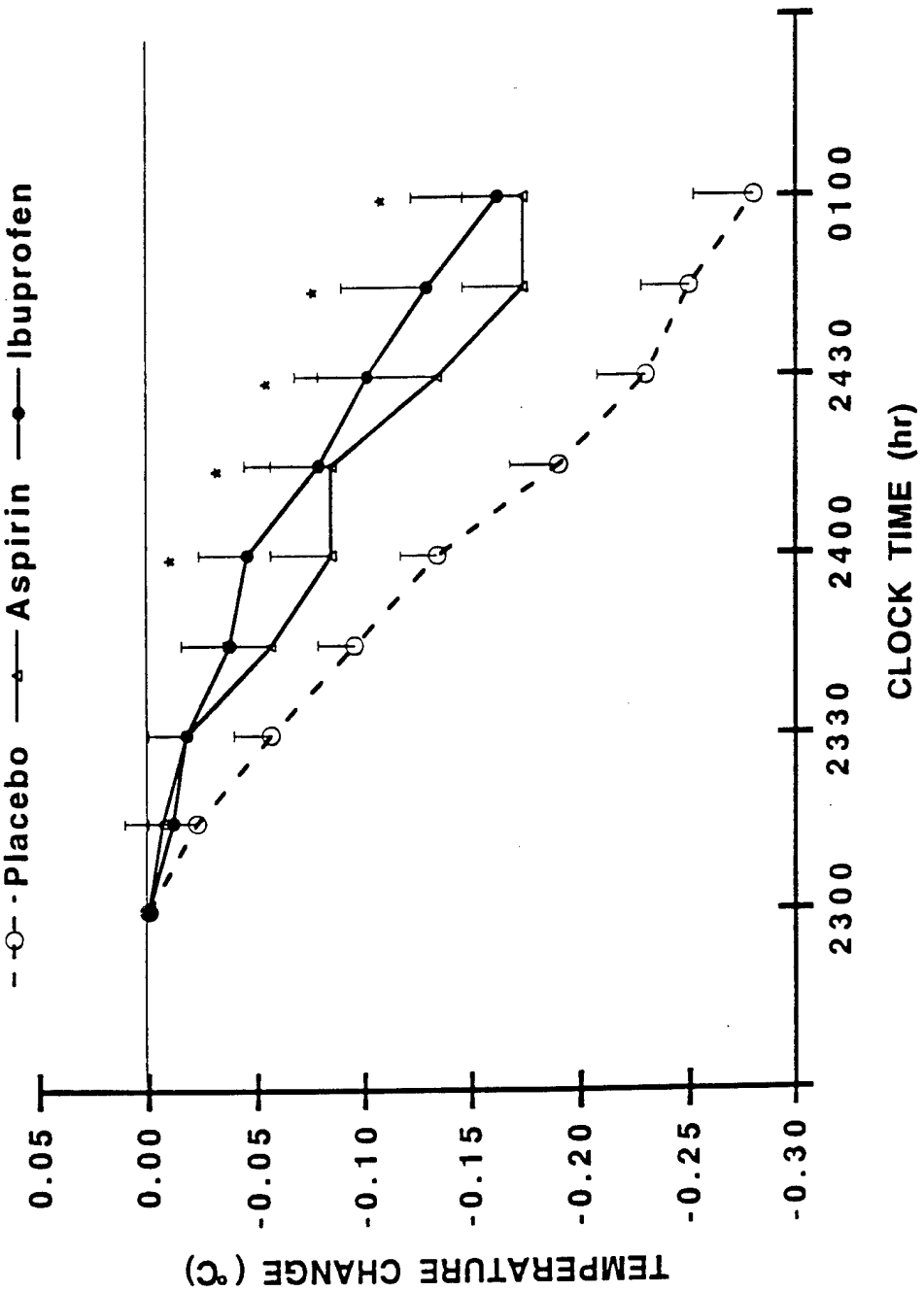

Placebo
Aspirin
Ibuprofen
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
TEMPERATURE CHANGE (°C)
2300
2330
2400
2430
0100
CLOCK TIME (hr)

Figure 25

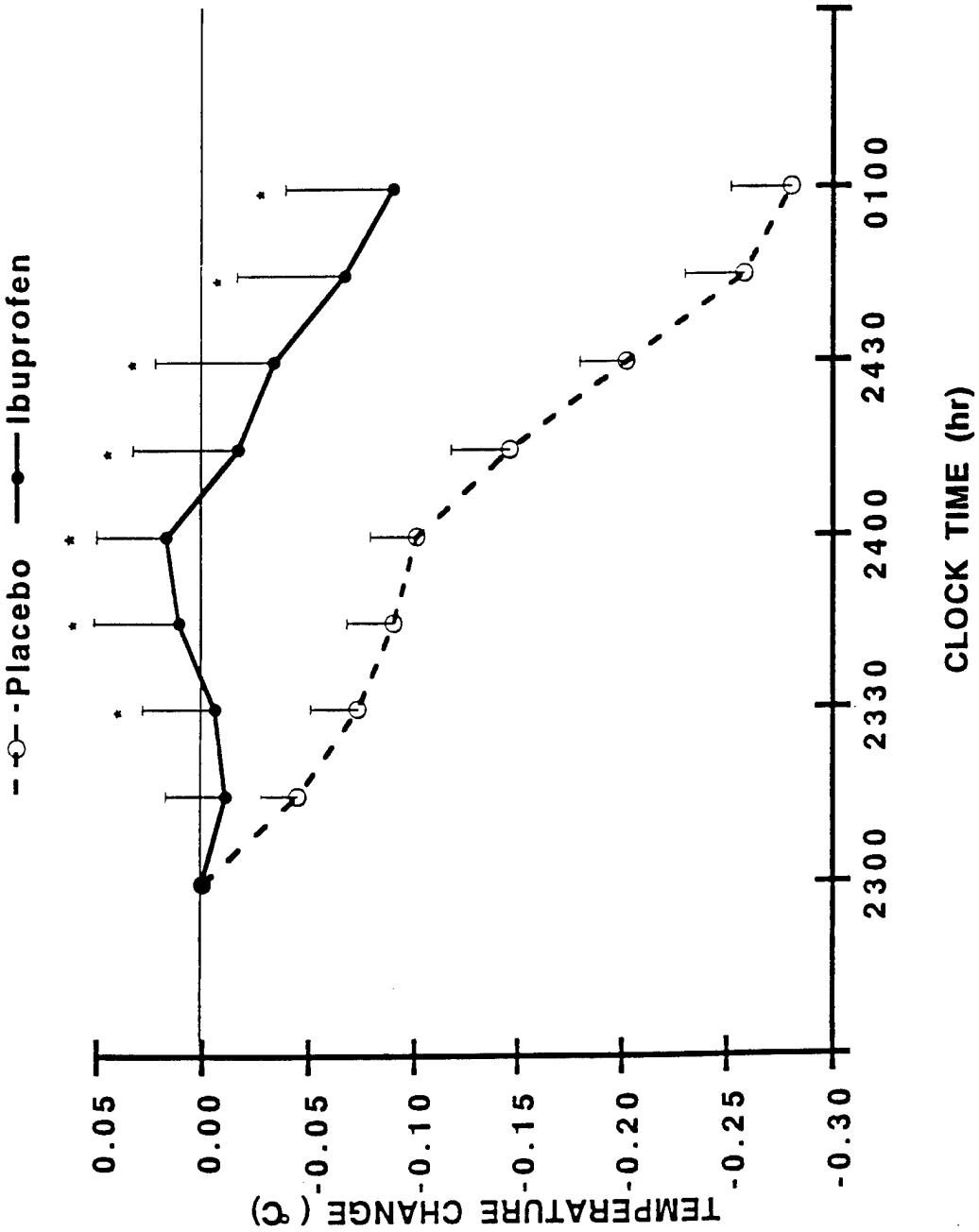

Placebo
Ibuprofen
TEMPERATURE CHANGE (°C)
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
2300
2330
2400
2430
0100
CLOCK TIME (hr)

Figure 26

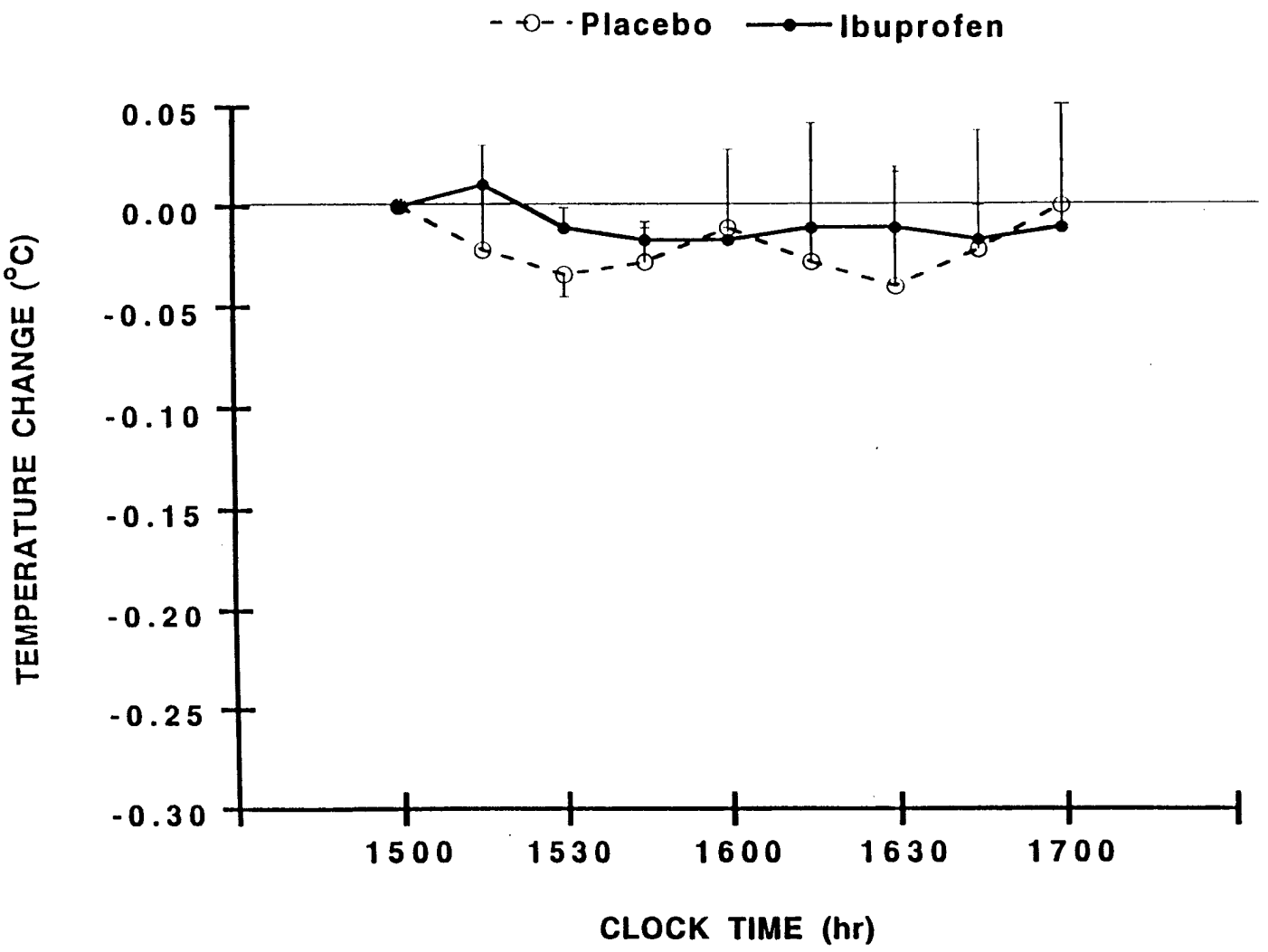

Placebo
Ibuprofen
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
TEMPERATURE CHANGE (°C)
1500
1530
1600
1630
1700
CLOCK TIME (hr)

Figure 27

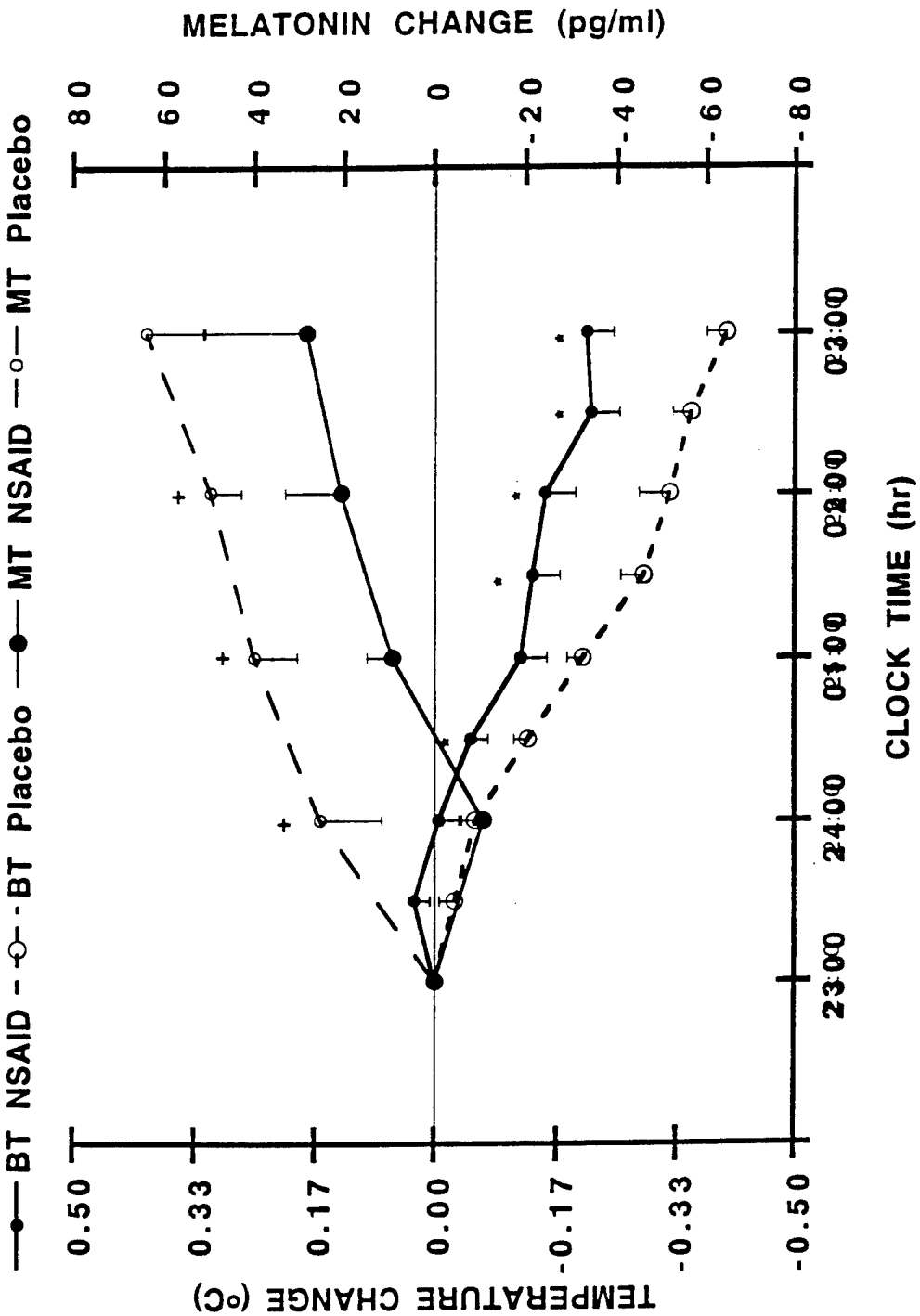

MELATONIN CHANGE (pg/ml)
80
60
40
20
0
-20
-40
-60
-80
BT NSAID
BT Placebo
MT NSAID
MT Placebo
0.50
0.33
0.17
0.00
-0.17
-0.33
-0.50
TEMPERATURE CHANGE (°C)
23:00
24:00
01:00
02:00
03:00
CLOCK TIME (hr)

Figure 28
Alpha EEG
(8-12 Hz)

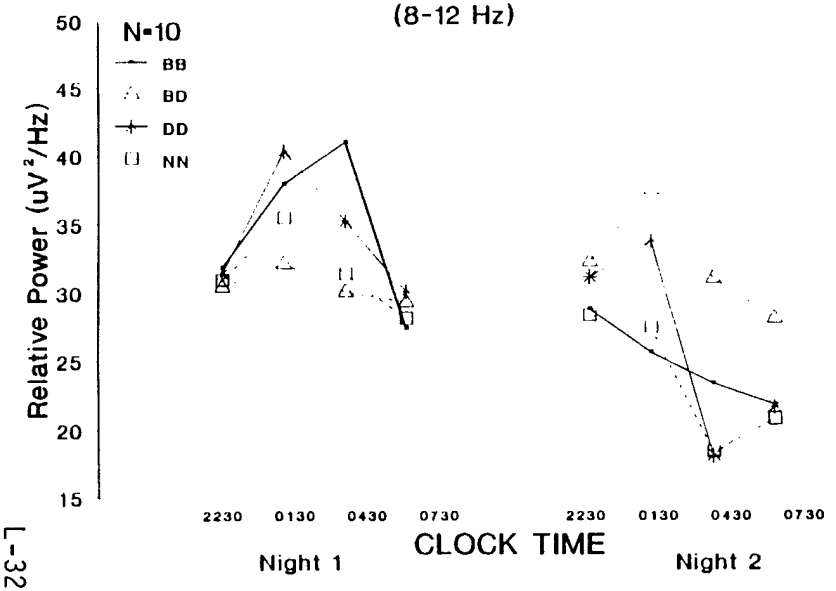


Figure 29
Theta EEG
(4-7 Hz)

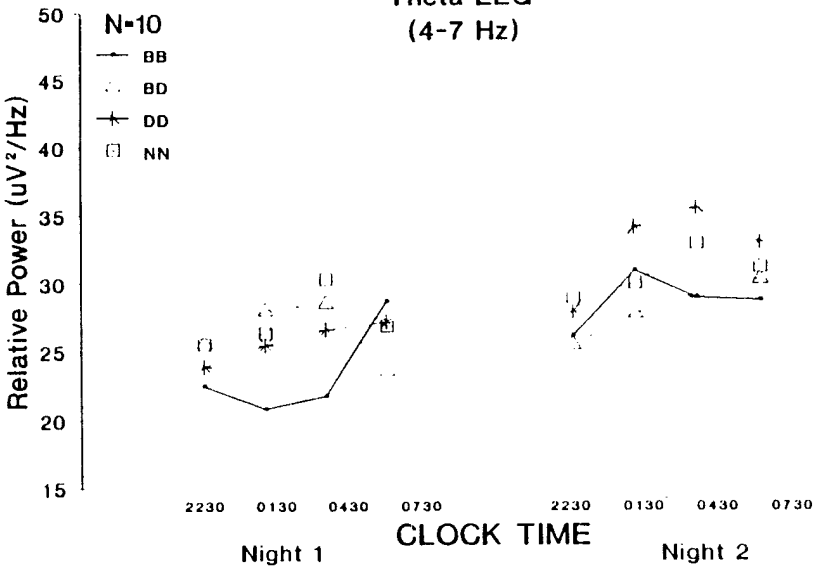


Figure 30
Maintenance of Wakefulness Test

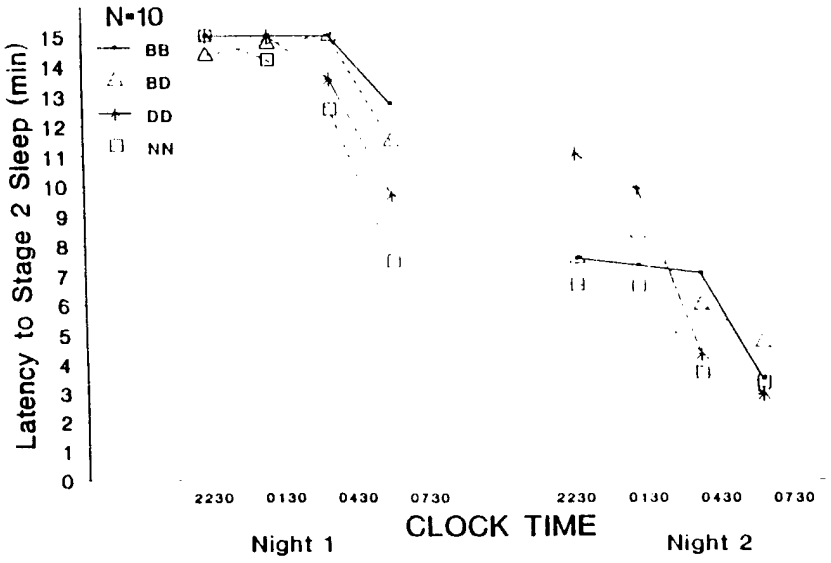


Figure 31
Stanford Sleepiness Scale

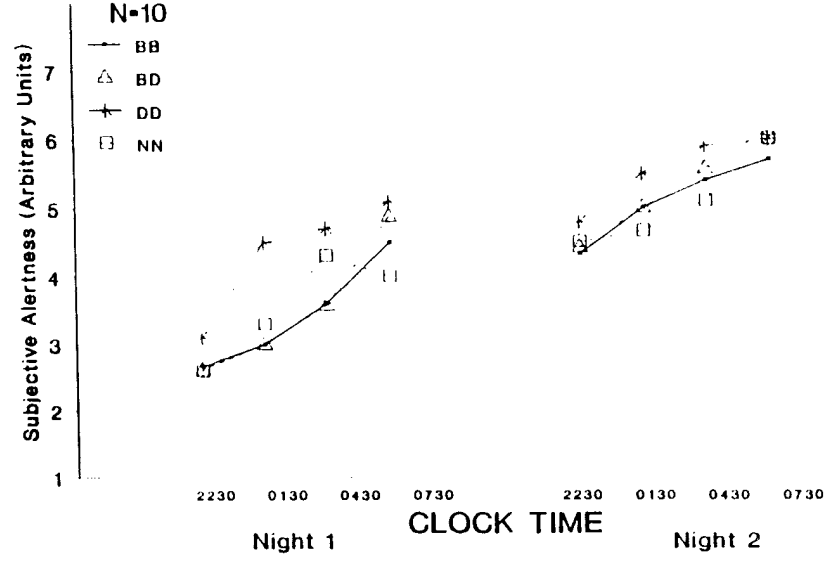


Figure 32
Continuous Recognition Task

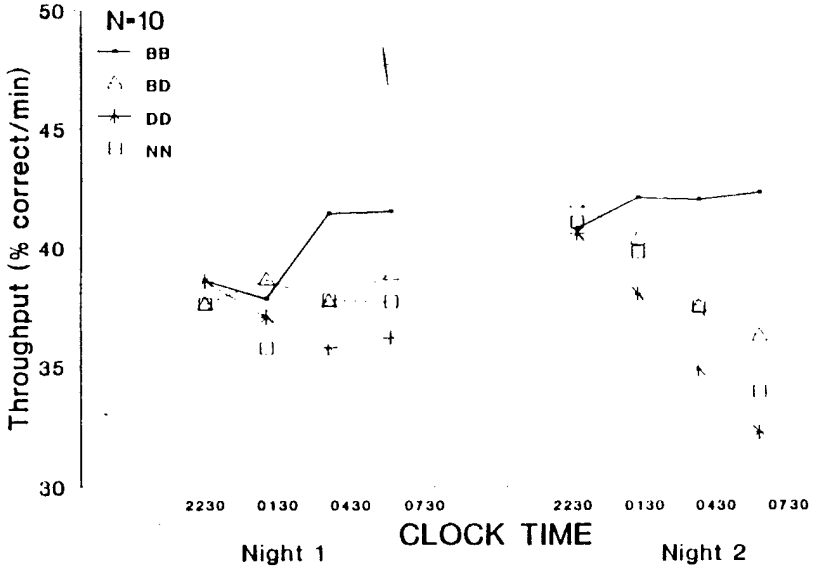


Figure 33
Dual Task

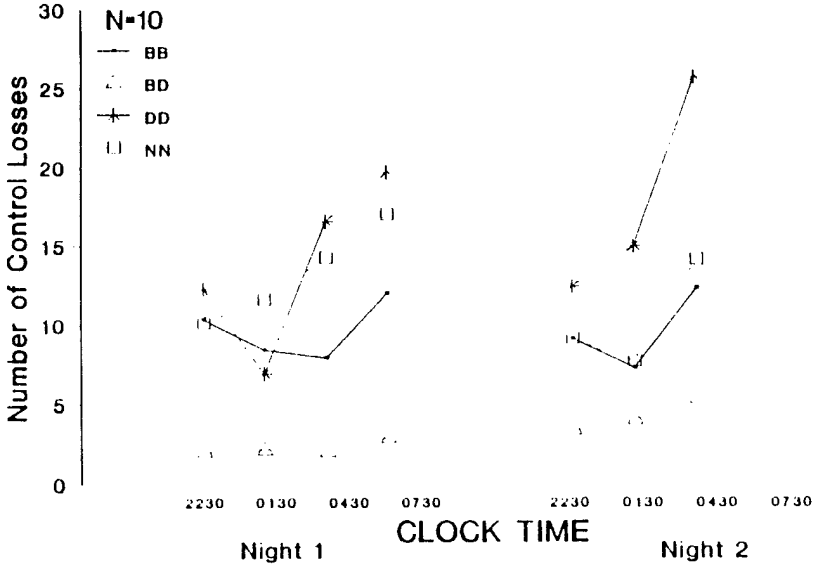


Figure 34
Dual Task

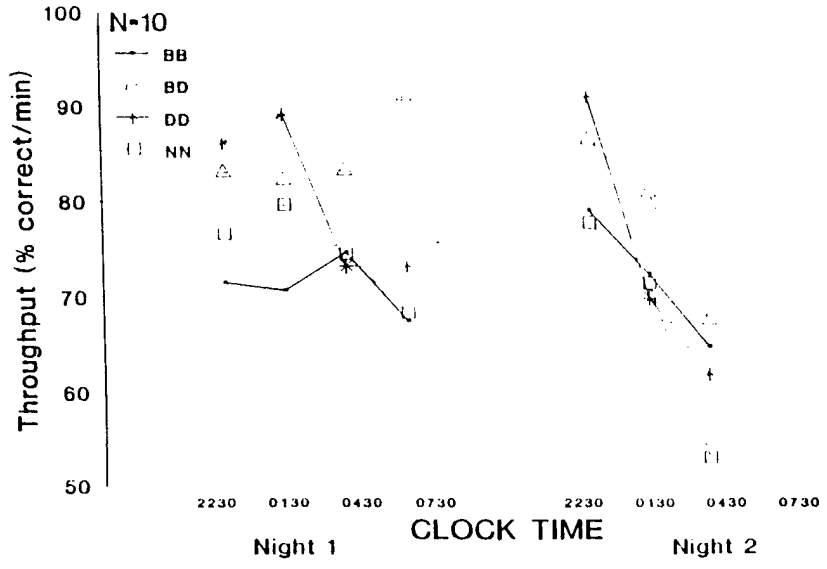


Figure 35
Probed Memory Recall Test

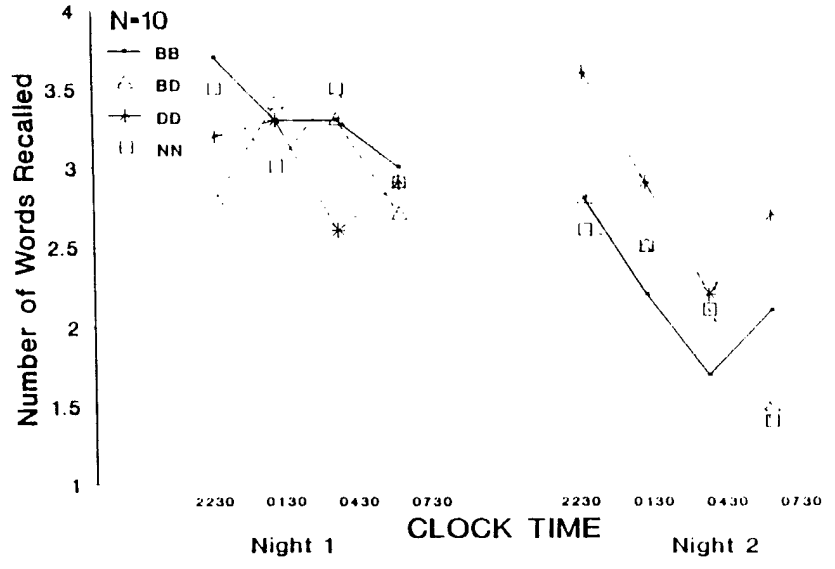


Figure 36
Procedural Memory Task (Basic)

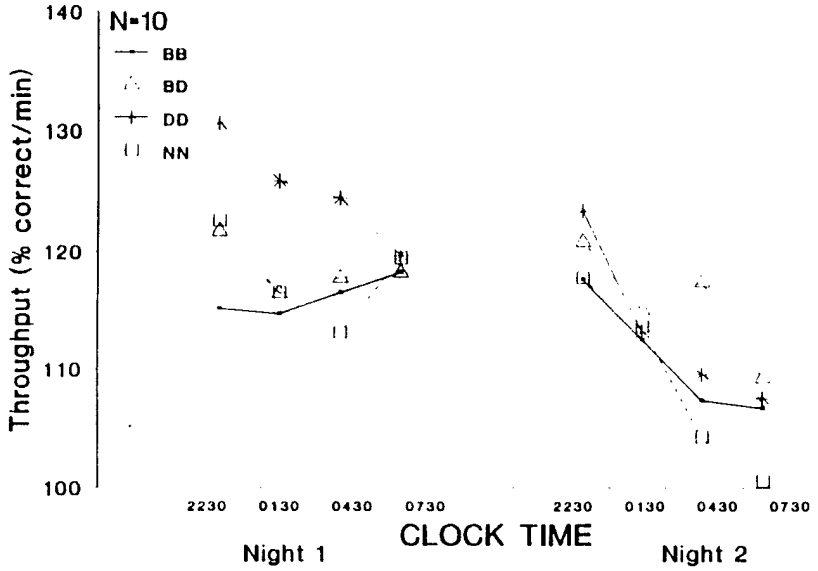


Figure 37
Procedural Memory Task (Coded)

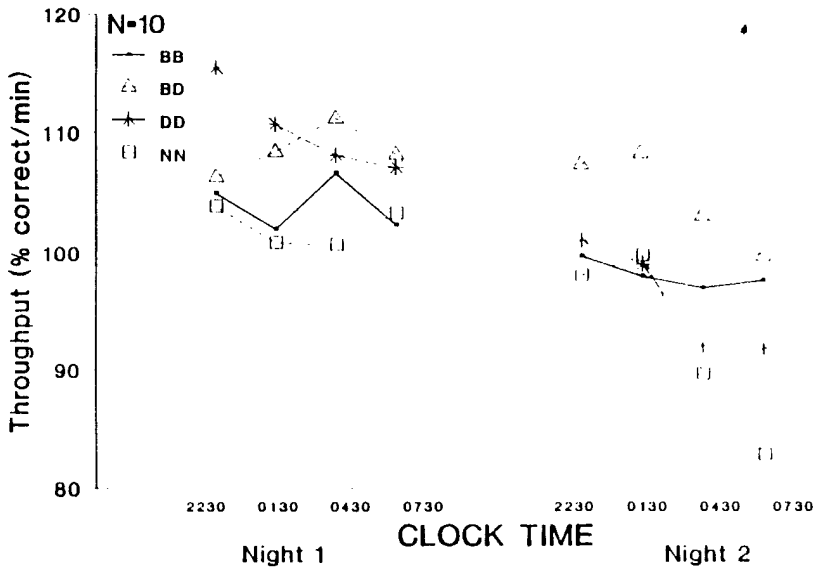


Figure 38
Switching Task (Mannequin)

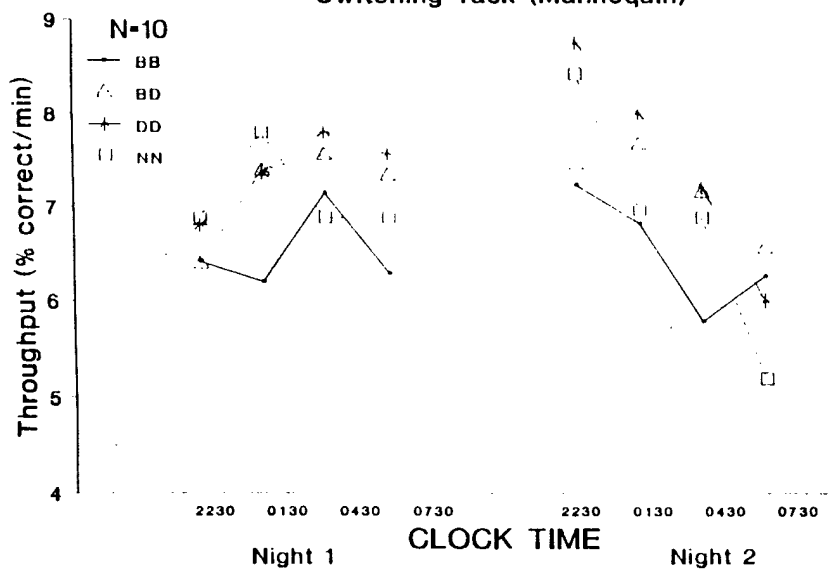


Figure 39
Switching Task (Processing)

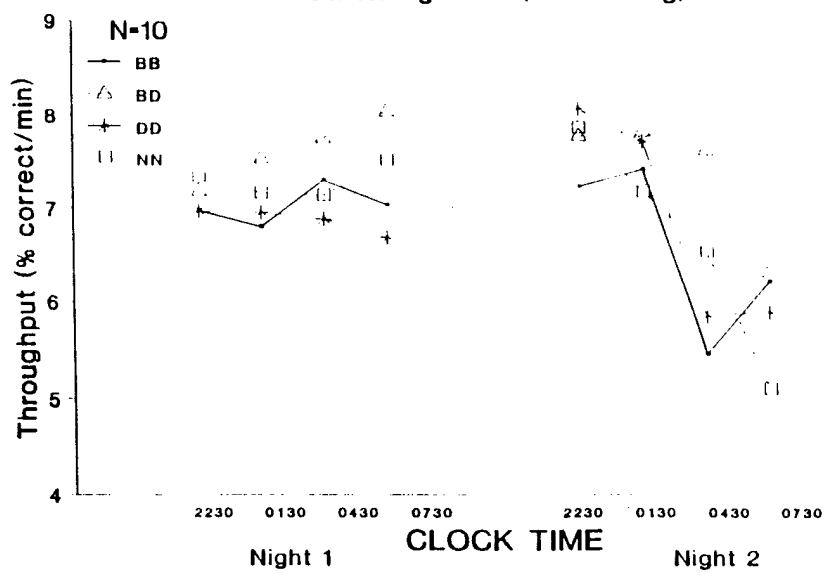
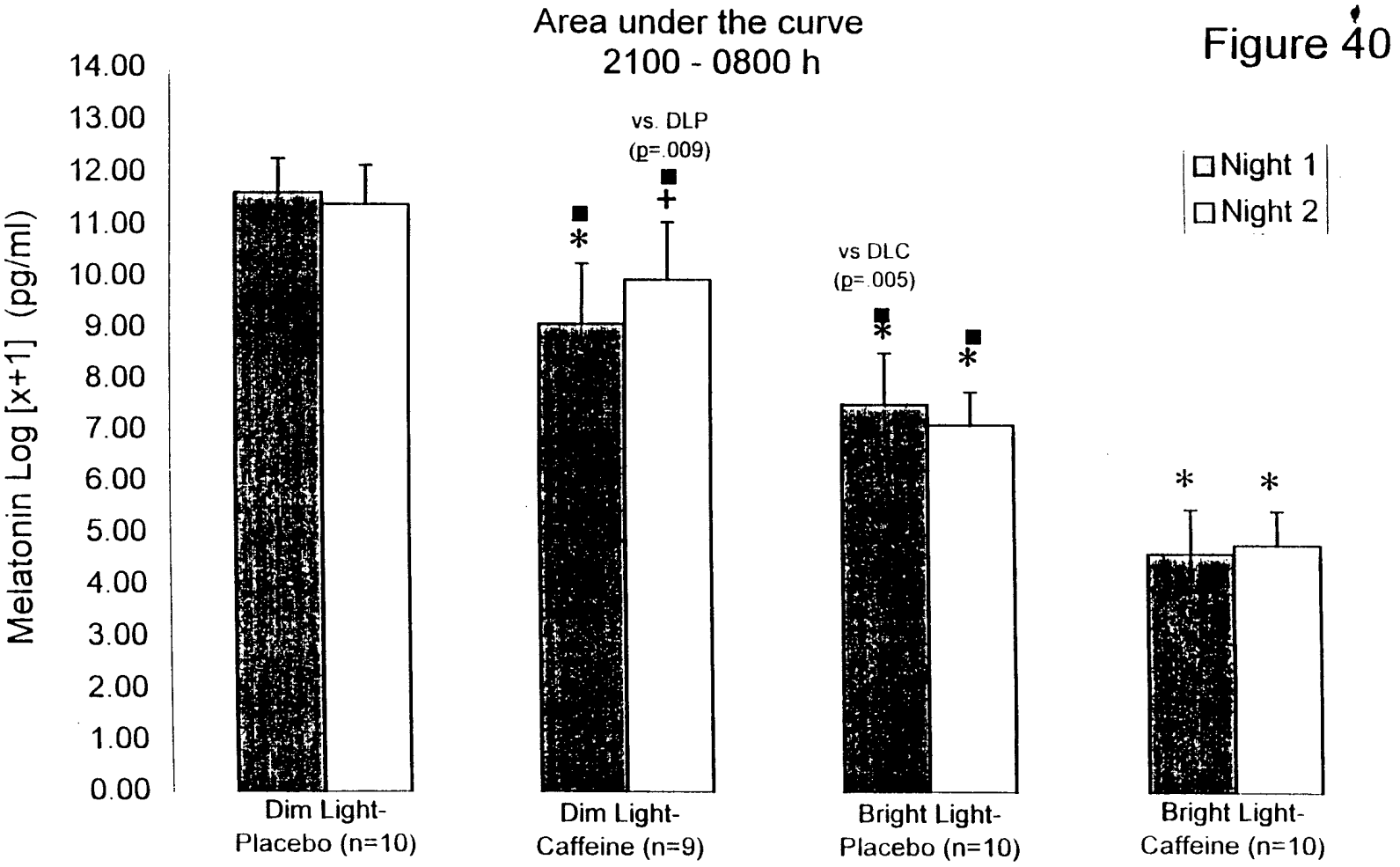


Figure 40

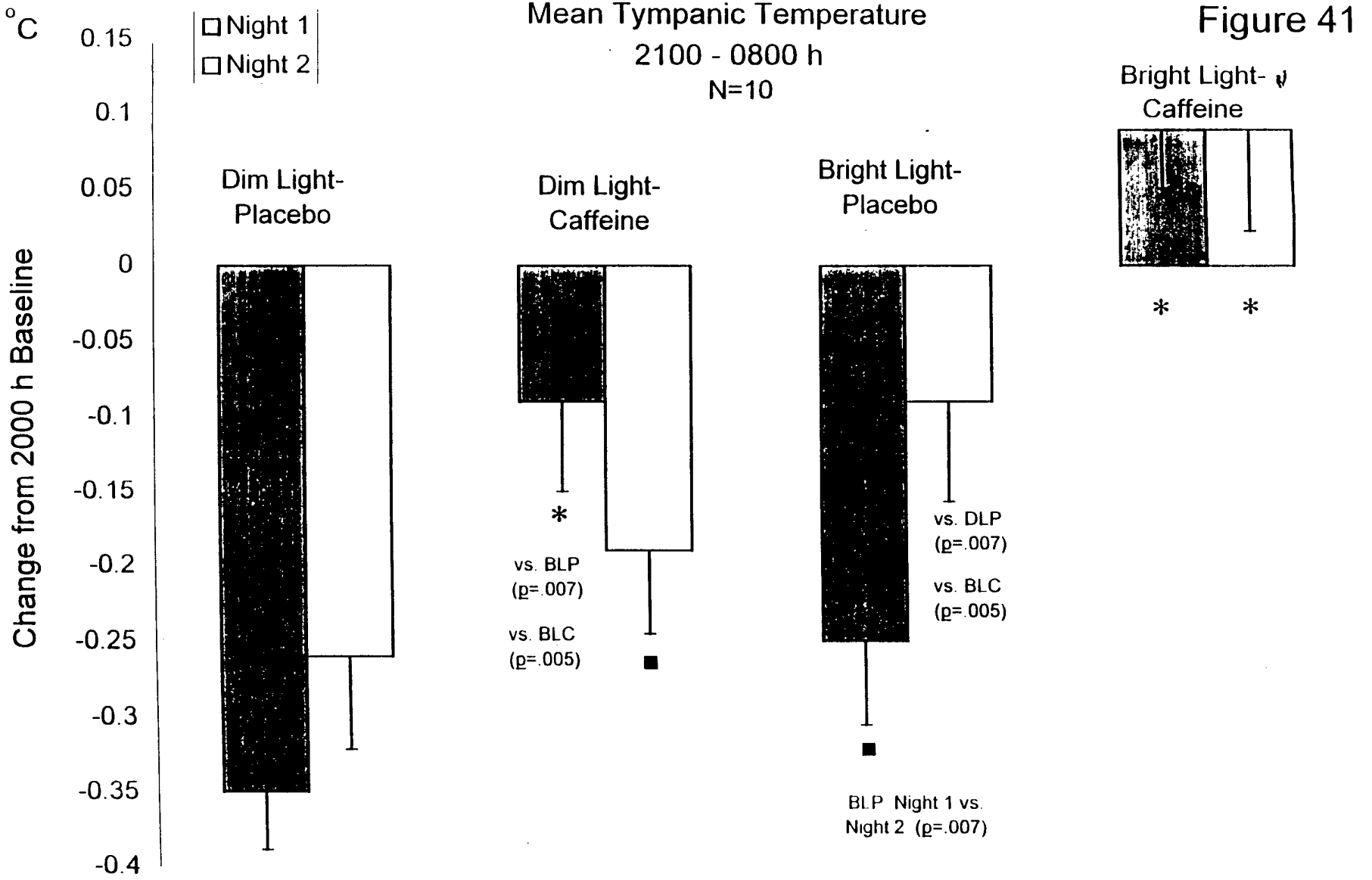


* =Significant difference from DLP (p < 003)
+ = Significant differernce between BLP & DLC (p < .003)
■= Significant difference between BLC & BLP or BLC & DLC (p < .003)

Trends noted as p values in parentheses

Bars denote Standard Error of the Mean

Figure 41



* = Signinificant difference from DLP (p < .003)

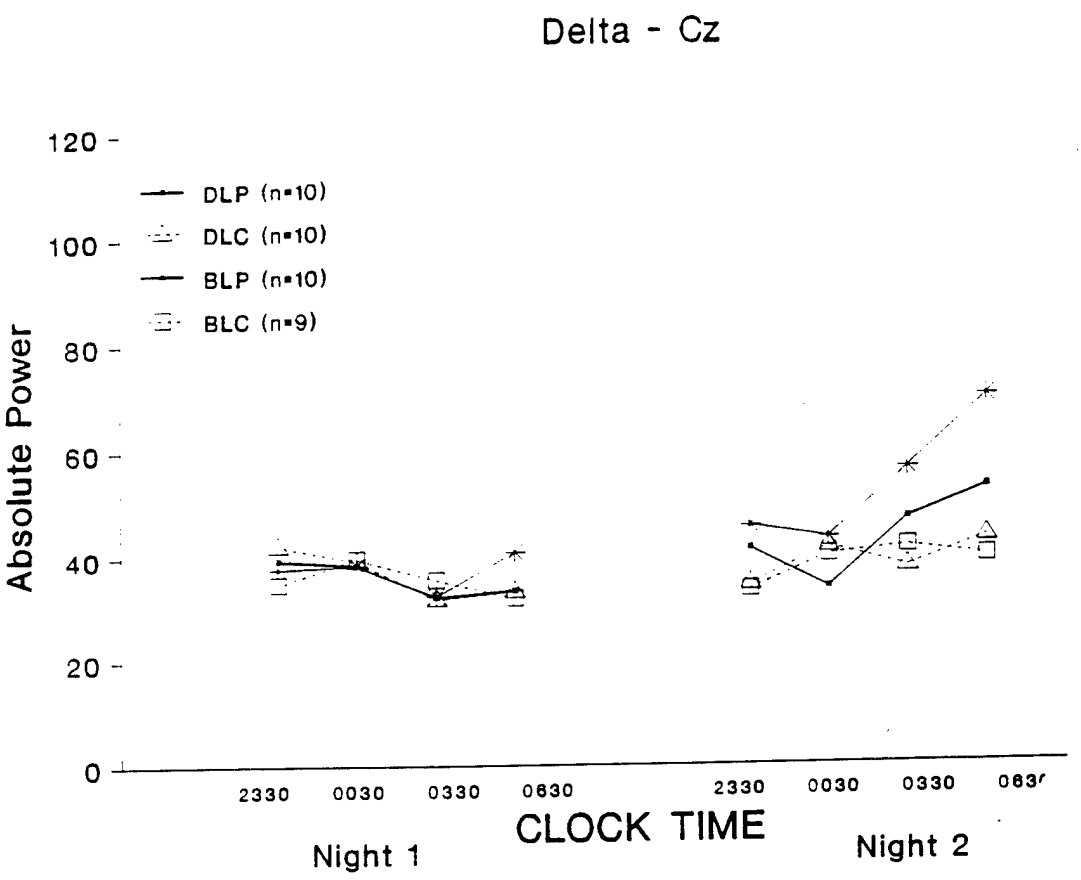
+ = Significant difference between DLC and BLP (p < .003)

■ = Significant difference between BLC & DLC or BLC and BLP (p < .003)

Trends noted as p values in parentheses

Bars denote Standard Error of the Mean

Figure 42


Delta - Cz
120
100
80
60
40
20
0
Absolute Power
DLP (n=10)
DLC (n=10)
BLP (n=10)
BLC (n=9)
2330
0030
0330
0630
2330
0030
0330
Night 1
CLOCK TIME
Night 2

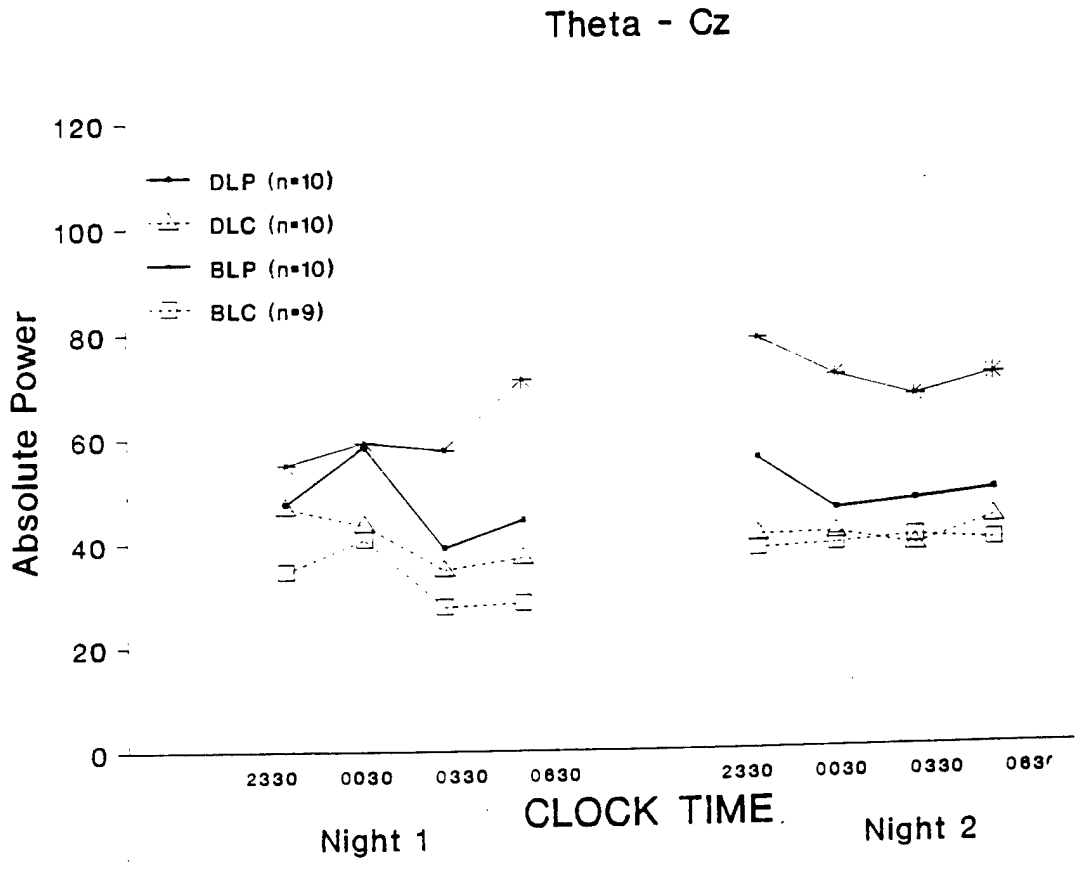
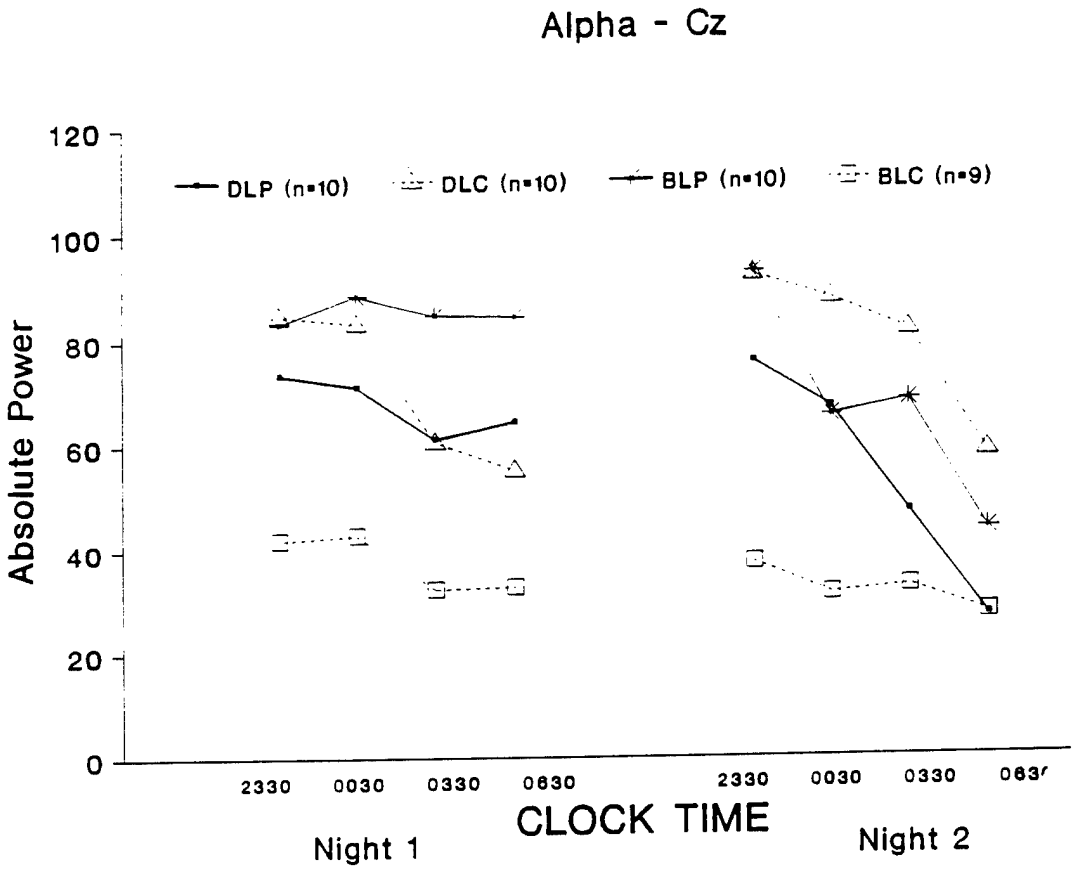
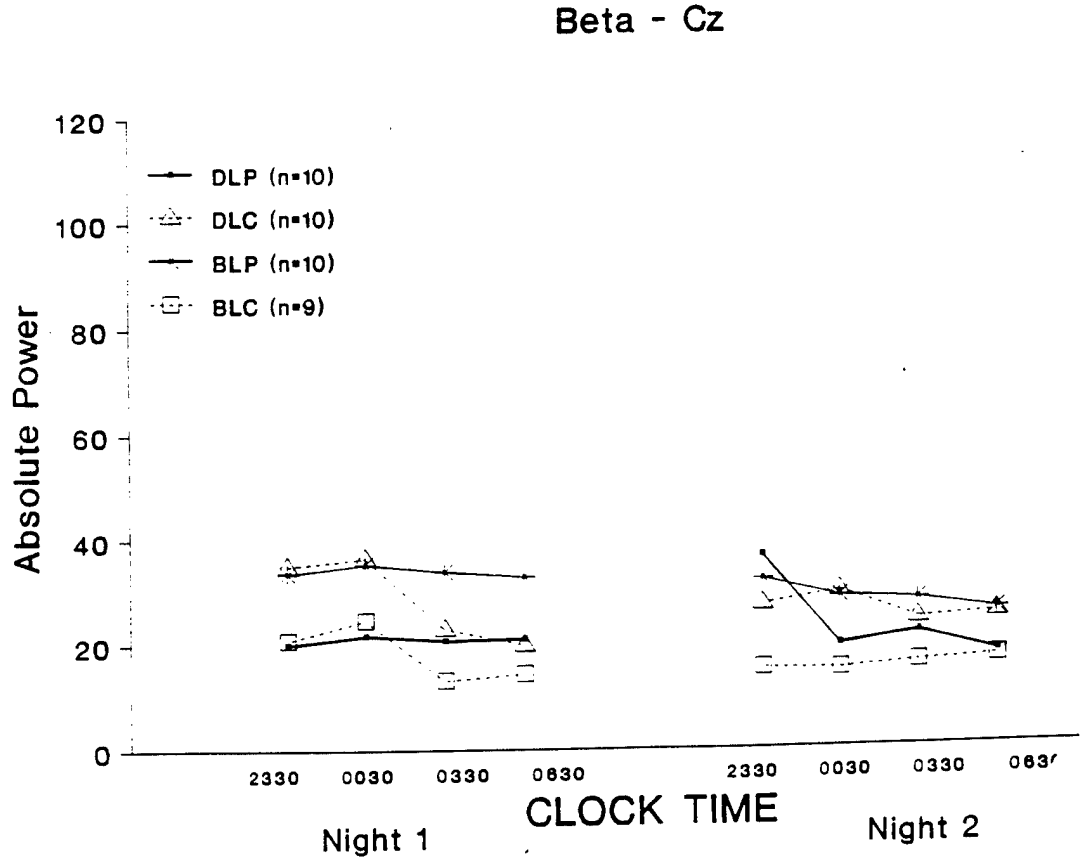

Theta - Cz
120
100
80
60
40
20
0
Absolute Power
DLP (n=10)
DLC (n=10)
BLP (n=10)
BLC (n=9)
2330
0030
0330
0630
2330
0030
0330
Night 1
CLOCK TIME
Night 2

Figure 43


Alpha - Cz
DLP (n=10)
DLC (n=10)
BLP (n=10)
BLC (n=9)
Absolute Power
120
100
80
60
40
20
0
2330
0030
0330
0630
2330
0030
0330
CLOCK TIME
Night 1
Night 2


Beta - Cz
DLP (n=10)
DLC (n=10)
BLP (n=10)
BLC (n=9)
Absolute Power
120
100
80
60
40
20
0
2330
0030
0330
0630
2330
0030
0330
CLOCK TIME
Night 1
Night 2